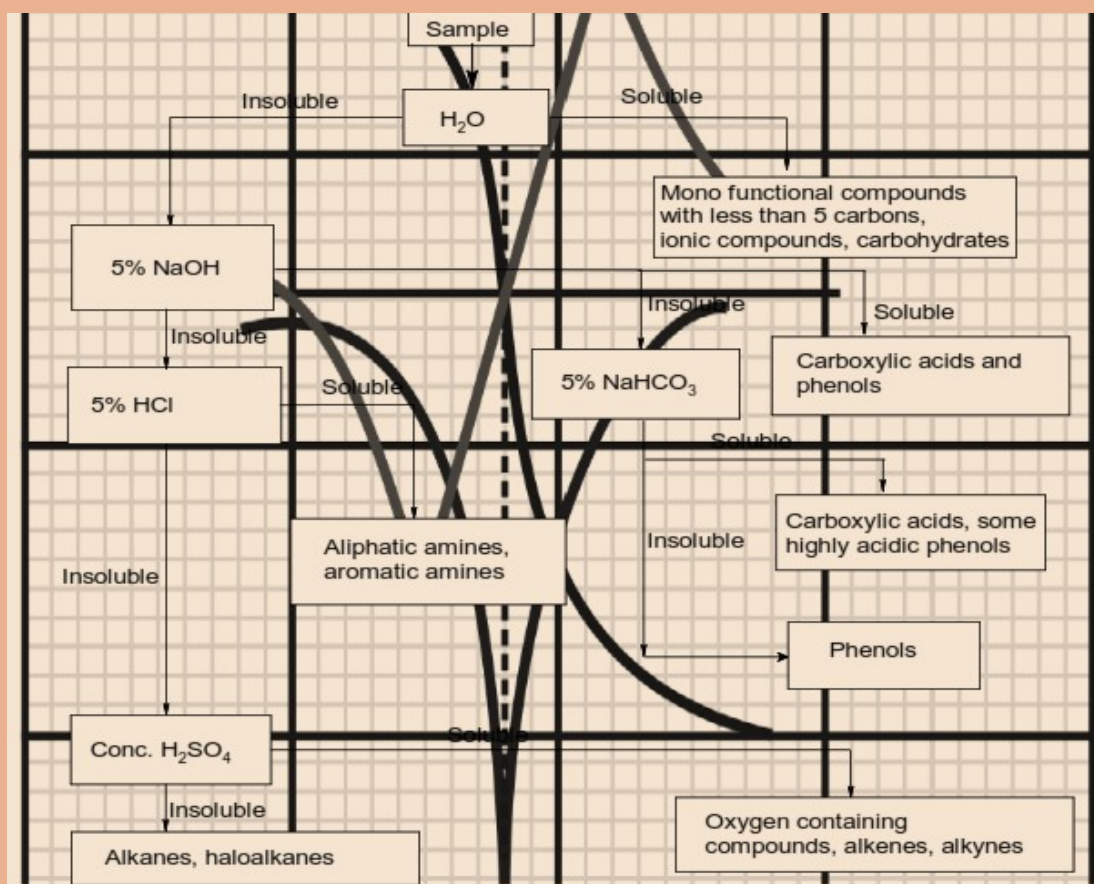


BCHCL-136

CHEMISTRY LAB-III: SOLUTIONS, PHASE EQUILIBRIUM, CONDUCTANCE, ELECTROCHEMISTRY AND FUNCTIONAL GROUP ORGANIC CHEMISTRY-II



BCHCL-136
**CHEMISTRY LAB III: SOLUTIONS,
PHASE EQUILIBRIUM,
CONDUCTANCE, ELECTROCHEMISTRY
AND FUNCTIONAL GROUP
ORGANIC CHEMISTRY-II**

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CHEMISTRY LAB III : SOLUTIONS, PHASE EQUILIBRIUM, CONDUCTANCE, ELECTROCHEMISTRY AND FUNCTIONAL GROUP ORGANIC CHEMISTRY-II

Welcome to the third Semester laboratory course in chemistry. This manual gives the procedural details of various experiments you are required to perform in the course of this lab work. Appropriate conceptual basis has also been laid for each experiment to make this manual complete in itself.

This Laboratory manual has two sections. **Section A** deals with experiments in physical chemistry and **Section B** with experiments in organic chemistry. There are seven experiments in the Section A. The first four experiments are based on chemical and phase equilibrium. Whereas, the last two experiments provide procedural detail for the conductometric and potentiometric titrations of acids and bases. These involve, the measurement of conductance and emf respectively as a function of volume of the titrant for the determination of the equivalence point of the titrations.

Part B mainly deals with qualitative organic analysis. We shall study the experiment procedures for the qualitative classification tests and preparation of derivatives for organic compounds having commonly encountered functional groups such as phenolic, carbonyl, carboxyl, amino, and nitro. Besides these three experiments based on organic qualitative analysis we have also include three more experiments which deal with tests use for differentiating reducing and non-reducing sugars, determination of glycine and action of salivary amylase on starch.

All the thirteen experiments of this course are to be performed in about six days.

Expected Learning Outcomes

After studying this course and performing the given experiments, you will be able to:

- determine the equilibrium constant for a reaction using distribution technique;
- construct the phase diagram for the naphthalene-benzoic acid system using cooling curves method;
- determine the CST of partially miscible liquid pairs such as phenol and water and study the effect of impurities on it;
- determine the dissociation constant of the given weak acid by plotting pH titration curve;
- calculate the strength of given HCl (or acetic acid) solution by perform conductometric (and potentiometric) titrations with NaOH
- describe the steps involved in the identification organic compounds by classical qualitative organic analysis;
- describe functional group tests and methods of the preparation of derivatives;
- identify the unknown organic compounds;
- carry out tests used for differentiating between reducing sugars and non-reducing sugars;
- carry out experiment for the determination of glycine;
- describe the reaction of salivary amylase with starch; and
- maintain laboratory records for the experiments performed during this course.

Study Guide

The general pattern of discussion of experiments in this course is similar to that of 1st and 2nd semesters Chemistry Labs. In this lab course, you would be doing thirteen experiments. In this course, the performance of the experiments, recording the data, calculations and expression of result are to be done as in earlier chemistry lab course. The maintenance of

Laboratory note book may be done as per the detail guidelines given for each Section. Some general guidelines for the maintaining Laboratory note book are given on next page. Remember that each experiment is going to be evaluated. Hence, read the Lab manual before going the laboratory for performing the experiments. This will help you in doing experiment in a proper way.

We want you to share the thrill of learning by doing. There is no better way to learn.

So, Best of Luck.

Laboratory Notebook

It is essential to keep a proper record of the work that has been done. The record should reflect all the observations at various stages of the experiment. The observations prove helpful in correct interpretation of an experimental result.

While preparing a laboratory note book, the following important points may be kept in mind.

- A bound note book should be used for laboratory record. You may use a laboratory record note book in which one side has ruled pages and other side is unruled.
- All entries must be made in ink. If you commit a mistake, it should be crossed and correct entry should be made.
- The first few pages in the note book should be left for making a list of contents.
- Graphs drawn should be attached to the laboratory note book.
- The laboratory note book is a complete record of all operations. Date, time, the number and the title of each experiment must be entered regularly.
- Record all observations and data in the note book at the time they are obtained. Never use scraps of paper for noting particulars like masses of substances weighed, melting points etc. They might get lost or mixed up.
- The record should be clearly written and well organised. On reading it, one should be able to understand what has been done.
- It may not be necessary to copy out the exact procedure, since this is given in your laboratory manual.
- The detailed calculations are to be shown.
- Results and conclusions drawn should be summarised for each experiment and explanation provided, if the results vary from those expected.

Certain marks have been allotted for maintaining a good laboratory note book too.

IMPORTANT

- **Attendance** is compulsory in the Laboratory Course work held generally at the Study Centre.
- The Laboratory Course is worth **2 credits** to be completed over **7 days** duration:
 - **6 days** of the **Guided** Laboratory work
 - **1 day** of the **Unguided** Laboratory work
- To successfully complete the laboratory course you will have to pass (at least **35% marks**) in the Guided and Unguided components separately.

EXPERIMENT 1

STUDY THE EQUILIBRIUM OF REACTIONS BY THE DISTRIBUTION METHOD

Structure

1.1 Introduction	1.4 Experiment 1b: To Study the Complex Formation between Copper Ions, Cu(II) And Ammonia and Determine the Formula of the Complex using Distribution Technique
Expected Learning Outcomes	$\text{Cu}^{2+} + 4\text{NH}_3 \rightarrow [\text{Cu}(\text{NH}_3)_4]^{2+}$
1.2 Theory of Nernst Distribution Law and Distribution Constant	Principle
1.3 Experiment 1a: To Determine the Equilibrium Constant for the Reaction using Distribution Technique	Requirements
$\text{KI} + \text{I}_2 \rightleftharpoons \text{KI}_3$	Procedure
Principle	Observations
Requirements	Calculations
Procedure	Result
Observations	1.5 Answers to SAQs
Calculations	
Result	

1.1 INTRODUCTION

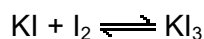
In the previous practical courses you have learnt the basics of the various organic, inorganic and physical practicals. In this laboratory course, you would be doing the physical practical experiments. Here you will have to recall the details of Nernst Distribution Law and Distribution constant in order to perform experiments based on this theory.

Expected Learning Outcomes

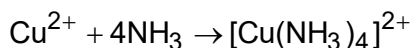
After performing the given experiments you should be able to:

- ❖ explain the principle of Nernst distribution law & Distribution constant;

- ❖ determine the equilibrium constant for the reaction using distribution technique:



- ❖ determine the formula of the complex formation between copper ions, Cu(II) and ammonia using distribution technique:



1.2 THEORY OF NERNST DISTRIBUTION LAW AND DISTRIBUTION CONSTANT

You have already studied the theory of Nernst Distribution Law and Distribution Constant in Unit 2, Section 2.4 of BCHCT 135. Please revisit the section to recall it. Here also the theory has been discussed briefly so that it will help you to perform experiments based on this theory.

Determination of Distribution Constant (K_D) and Nernst Distribution Law

When a solid or a liquid is shaken up with a mixture of two immiscible liquids (A and B) and if the solute is soluble in both the liquids at a given temperature, then it distributes between two liquids in a definite manner so that the ratio of its concentrations in the two solvents is independent of the amount of the solute distributed between the two phases, provided the solute is in the same molecular state in both the solvents.

Thus, $K_D = \left(\frac{C_A}{C_B} \right)$ where C_A and C_B are the equilibrium molar concentration of solute in the two liquids and K_D is a constant known as distribution or partition coefficient. This law is known as '**Nernst Distribution Law**' or **Partition Law**. The value of K_D depends on temperature of the system and does not depend on the relative amount of the two layers and also that of the solute.

In the Unit 2 of BCHCT 135, you have also learnt about the limitations of distribution law. So you have studied that it is valid only when the solute undergoes no change such as dissociation or association in either of the solvents.

SAQ 1

What are the limitations of the Nernst Distribution Law?

There may be different situations for the systems of liquids (A & B) and the solute. Let us discuss them one by one. In these cases we take ' C_A ' as total concentration of undissociated solute in liquid A, ' C_B ' the total concentration of undissociated solute in liquid B and α_B as the degree of dissociation of solute molecules in liquid B.

Let us consider first the situation where the solute behaves normally in liquid A but dissociates in liquid B:

$$K_D = \left(\frac{C_A}{C_B(1-\alpha_B)} \right) \quad \dots(1.1)$$

There may be situation where solute dissociates in both liquids A and B but to different extents.

$$K_D = \left(\frac{C_A(1-\alpha_A)}{C_B(1-\alpha_B)} \right) \quad \dots(1.2)$$

Whenever, solute behaves normally in liquid A but undergoes association in liquid B, then 'C_A' would be total concentration of solute in liquid A and 'C_B' be the total concentration of solute in liquid B. Let in liquid B, the solute molecules undergoes association as $nx \rightleftharpoons X_n$ and thus in liquid B, you would not only find concentration of single molecules but the associated molecules also. Let 'α_B' be the degree of association of solute molecules in liquid B.

$$K_D = \left(\frac{C_A}{n\sqrt{C_B(1-\alpha_B)}} \right) \quad \dots(1.3)$$

Lastly, if solute undergoes dissociation in liquid A and association in liquid B then,

$$K_D = \left(\frac{C_A(1-\alpha_A)}{n\sqrt{C_B(1-\alpha_B)}} \right) \quad \dots(1.4)$$

Thus you see, we have dealt with all the situations for the systems (liquid A & B) and the solute. Next, you are going to learn and perform experiments based on this.

SAQ 2

What would be the distribution coefficient (K_D) when the solute behaves normally in liquid A but association in liquid B?

1.3 EXPERIMENT 1a: TO DETERMINE THE EQUILIBRIUM CONSTANT FOR THE REACTION USING DISTRIBUTION METHOD

$KI + I_2 \rightleftharpoons KI_3$

Now you are going to learn and thereby perform experiments based on the distribution law.

1.3.1 Principle

In aqueous solutions, iodine (I₂) dissolves sparingly. Also, iodine may volatilise from them. Whereas, I₂ reacts with potassium iodide (KI) in aqueous solution to form the potassium triiodide complex (KI₃) which yields I₂ easily when the equilibrium is disturbed.

In the previous practical course BCHCL 132, in the Experiment 5, you have learnt details of iodometry, which you should remember here.

Iodine is available in a sufficiently pure state, but it is very slightly soluble in water. Therefore, its standard solution in pure water cannot be prepared. However, iodine gets dissolved easily in water containing potassium iodide forming triiodide complex which is unstable:



Now let us discuss the two sources of error in iodometric titrations. They are the following:

- (i) Iodine is a little bit volatile in solution, and therefore these titrations should not be performed in hot solutions.
- (ii) In presence of light, iodide ion (from KI), is oxidized by the oxygen of air:



Therefore, water used for dissolving potassium iodide, which is used to dissolve iodine, should be boiled to remove dissolved oxygen.

To determine the equilibrium constant for the reversible reactions given in Eq. 1.5, the distribution coefficient of the iodine, K_D , between water and organic solvent such as carbon tetrachloride (CCl_4) is determined by titrating aliquots of the two layers with standard sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) solution.

$$K_D = \frac{c_{\text{I}_2(\text{org})}}{c_{\text{I}_2(\text{aq})}} \quad \dots(1.7)$$

K_D is thus determined using both aqueous (water) and organic (CCl_4) layer, the I_2 is then shaken with aqueous potassium iodide (KI) solution and CCl_4 . The mixture is then allowed to stand until equilibrium is established. At equilibrium, the aqueous layer contain free iodine, free potassium iodide and potassium triiodide complex.

The concentration of I_2 in the two layers (i.e. aqueous and organic), are determined by titration with standard $\text{Na}_2\text{S}_2\text{O}_3$ solution. The equilibrium constant (K) is given by

$$K = \frac{[\text{KI}_3]}{[\text{KI}][\text{I}_2]} \quad \dots(1.8)$$

Where, $[\text{KI}_3]$, $[\text{KI}]$ and $[\text{I}_2]$ represent the equilibrium concentrations in any single aqueous layer.

Since, the distribution law applies only to species common to both layers, the concentration of free iodine in the aqueous potassium iodide layer, $c_{\text{I}_2(\text{aq})}$, can be determined from concentration of iodine in the organic layer, $c_{\text{I}_2(\text{org})}$, even when other species are present. By using the Eq. (1.8) we get,

$$c_{\text{I}_2(\text{aq})} = \frac{c_{\text{I}_2(\text{org})}}{K_D} \quad \dots(1.9)$$

1.3.2 Requirements

ApparatusStoppered bottles (200 cm³) - 8

Dropper -1

Burette– 1

Volumetric flask (250 cm³) – 4Conical flask (250 cm³) -1Pipette (20 cm³) – 1Measuring cylinder (10 cm³) -1

wash bottle for distilled water -1

Weighing bottle -1

Funnel (small) -1

Volumetric flask (1000 cm³) -1

Burette stand -1

ChemicalsI₂, KIDistilled H₂ONa₂S₂O₃K₂Cr₂O₇CCl₄

Starch (indicator)

Dilute H₂SO₄ acid

Solutions Provided: Procedures for the preparation of these solutions are given for the sake of information. These solutions would be prepared for you by the counselor.

Sodium thiosulphate solution (approx. M/5):

About 50 g of sodium thiosulphate crystals are dissolved in 1 dm³ of water that has been recently boiled and cooled. An amount of 0.4 g of sodium bicarbonate is added as a preservative and the solution stored in a clean bottle. Sodium thiosulphate solutions are somewhat unstable. Apart from oxygen and dissolved CO₂ they are easily attacked by air-borne bacteria with the liberation of sulphur. In case any turbidity is observed, the solution should be discarded.

Starch Solution: About 150 cm³ distilled water is heated to boiling in a beaker. Allowing this to be heated, stir 0.5 g to 1 g of soluble starch with about 10 cm³ of distilled water to make a paste. The paste is stirred into the boiling water and boiled gently for a few minutes and cooled. The solution should be almost clear. It is kept in a stoppered bottle. (Starch solution should be freshly prepared before use).

Potassium Iodide Solution: Prepared by dissolving 5.0 g KI in 100 cm³ of distilled water.

1.3.3 Procedure

(I) Preparation of standard potassium dichromate solution:

You have already done this in BCHCL 132 under the experiments of Titrimetry. Please revisit the exercise for recapitulation.

Prepare M/30 standard $K_2Cr_2O_7$ solution to standardise $Na_2S_2O_3$ solution. Take approximate mass of a glass weighing bottle. Then weigh it accurately with about 2.45 g of potassium dichromate. Transfer the salt to a clean and dry volumetric flask of 250 cm^3 capacity through a glass funnel. Find out the accurate mass of the bottle after transferring potassium dichromate. The difference between the two masses gives the actual amount of potassium dichromate transferred. Record these values in your observation note book. Finally, make the volume up to the mark by adding distilled water carefully.

Standardisation of sodium thiosulphate solution: Pipette 20 cm^3 of potassium dichromate solution in a 250 cm^3 conical flask, add 10 cm^3 of dilute sulphuric acid and 1 g sodium hydrogen carbonate with gentle swirling to liberate carbon dioxide. Sodium hydrogen carbonate maintains an atmosphere of CO_2 in the solution which displaces the air and prevents the oxidation of iodide from air. The reaction $4I^- + O_2 + 4H^+ \rightleftharpoons 2H_2O + 2I_2$ is catalysed by light, heat and acids. Then add 0.5 g potassium iodide or 10 cm^3 of 5% KI solution, swirl, cover the flask with a watch glass and allow the solution to stand for 5 minutes in a dark place. Titrate against sodium thiosulphate solution from the burette until a light pale yellow colour of iodine appears. Then add 2 cm^3 starch solution and continue to titrate until the blue colour of starch-iodine complex disappears on addition of a drop of the titrant. The final solution will be green coloured because of the presence of chromium (III) ions. Record the burette readings before and after the titration in Observation Table I. Repeat the same exercise to get at least two concordant readings.

Prepare 250 cm^3 of M/5 $Na_2S_2O_3$ solution. From this M/5 $Na_2S_2O_3$, prepare M/50 and M/500 $Na_2S_2O_3$ solution by dilution method.

Preparation of 250 cm^3 of M/50 $Na_2S_2O_3$ solution by dilution method for organic layer:

$$\frac{1}{5} \times V_{Na_2S_2O_3} = \frac{1}{50} \times 250$$

$$V_{Na_2S_2O_3} = \frac{\frac{1}{50} \times 250}{\frac{1}{5}} = 25 cm^3$$

25 cm^3 of M/5 $Na_2S_2O_3$ should be added to 225 cm^3 of distilled water in a 250 cm^3 volumetric flask.

(II) Determination of distribution coefficient (K_D) of iodine between water and CCl_4

- By taking four 200 cm^3 stoppered bottles, label them as 1, 2, 3 and 4 and prepare different systems of H_2O , I_2 and CCl_4 .
- Pipette out 30 cm^3 , 40 cm^3 , 50 cm^3 and 60 cm^3 of CCl_4 in different bottles. Also in each bottle, add 1g of iodine (I_2). Shake the bottles to dissolve iodine completely.
- Add 60 cm^3 , 50 cm^3 , 40 cm^3 and 30 cm^3 of H_2O in different bottles.

Table 1.1

Reagent Bottle Number	I ₂ (g)	CCl ₄ (cm ³)	H ₂ O (cm ³)
1	1	30	60
2	1	40	50
3	1	50	40
4	1	60	30

- (d) Stopper the bottles and shake vigorously for nearly an hour and then allow the bottle to stand until contents of each bottle separate in two distinct layers.

Titration of organic layer: Pipette out 5 cm³ of organic layer (CCl₄ layer/bottom layer), add 20 cm³ of water and a pinch of KI and keep it in dark for some time and then add 5 drops of starch as indicator and titrate against M/50 Na₂S₂O₃ solution till the end point *i.e.* when the blue colour disappears and note the reading. Record the burette readings before and after the titration in observation Table II. Repeat the same exercise to get at least two concordant readings.

Titration of aqueous layer: Pipette out 25 cm³ of aqueous layer (upper layer), add 5 drops of starch indicator and titrate against M/500 Na₂S₂O₃ taken in the burette till the end point *i.e.* disappearance of blue colour and note the reading [solubility of I₂ in H₂O is less that is why take large amount of water *i.e.* 25 cm³ and dilute Na₂S₂O₃ is used for titration]. Record the burette readings before and after the titration in observation Table III. Repeat the same exercise to get at least two concordant readings.

Tabulate the observation and calculate K_D .

(III) To Study the triiodide equilibrium:

Take four 200 cm³ stoppered bottles and label them as 5, 6, 7 and 8. In each bottle prepare different systems of aqueous KI, I₂, CCl₄. Then perform a similar experiment by just using a solution of KI in water (0.1 M KI) in place of water. Obtain the concentration of I₂ in two layers *i.e.* aqueous KI solution layer and organic CCl₄ layer by titrating them with standardized Na₂S₂O₃ solution.

Table 1.2

Reagent Bottle Number	I ₂ (g)	CCl ₄ (cm ³)	Aqueous solution of 0.1 M KI (V _{KI})
5	1	30	60
6	1	40	50
7	1	50	40
8	1	60	30

Remember that, the distribution applies only to a species common to both layers, the concentration of free iodine in the aqueous KI layer, $aq\ c_{I_2(org)}$ or C_2 , can be determined from concentration of iodine in the organic layer, $c_{I_2(org)}$ or

C_1 , even when other species are present. Thus, $c_{I_2(\text{org})}$ can be calculated as follows:

$$c_{I_2(\text{org})} = \frac{K_D}{c_{I_2(\text{aq})}} \quad \dots (1.10)$$

The total concentration of iodine, i.e. free (I_2) as well as that of combined with potassium iodide (KI_3) will be obtained from the titration of the aqueous KI layer with sodium thiosulphate solution.

Calculated concentration of free iodine = concentration of I_2 - concentration of KI_3 .

The concentration of KI_3 in the aqueous layer = the concentration of combined KI.

Initial concentration of KI in the aqueous solution of KI - concentration of combined KI = equilibrium concentration of free KI.

1.3.4 Observations

Mass of the weighing bottle	= $m_1 = \dots\dots\dots\text{g}$
Mass of bottle + potassium dichromate crystals	= $m_2 = \dots\dots\dots\text{g}$
Mass of the bottle (after transferring $K_2Cr_2O_7$)	= $m_3 = \dots\dots\dots\text{g}$
Mass of potassium dichromate transferred	= $m_2 - m_3 = m = \dots\dots\dots\text{g}$
	= $\dots\dots\dots\text{g}$
Molar mass of potassium dichromate	= $294.19 \text{ g mol}^{-1}$
Volume of $K_2Cr_2O_7$ prepared (V)	= 250 cm^3
Molarity of $K_2Cr_2O_7$ solution	= M_1
	= $\frac{m \times 4}{\text{Molar mass}} \text{ mol dm}^{-3}$
	= $\frac{m \times 4}{294.19}$
	= $\dots\dots\dots \text{mol dm}^{-3}$

Observation Table I

Potassium dichromate solution vs. sodium thiosulphate solution

Sl. No.	Volume of $K_2Cr_2O_7$ solution (cm^3)	Burette reading		Volume of $Na_2S_2O_3$ solution (cm^3) (Final - Initial)
		Initial	Final	
1.	20			
2	20			
3	20			

Observation Table II

Titration of M/50 $\text{Na}_2\text{S}_2\text{O}_3$ vs Organic Layer

Reagent Bottle Number	Burette reading for organic layer		Volume of $\text{Na}_2\text{S}_2\text{O}_3$ used $V_b = (V_2 - V_1) \text{ cm}^3$
	Initial (V_1)	Final (V_2)	
5			
6			
7			
8			

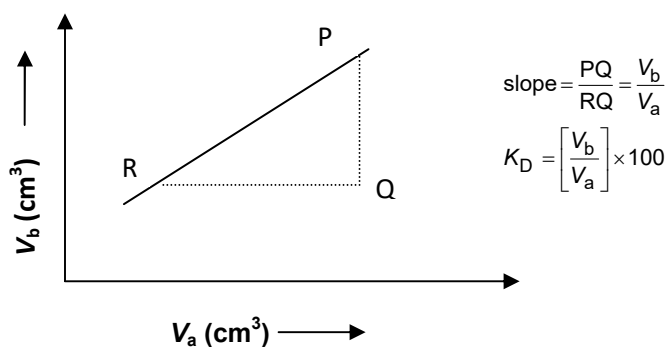
Observation Table III

Titration of M/500 $\text{Na}_2\text{S}_2\text{O}_3$ vs Aqueous Layer:

Reagent Bottle Number	Burette reading for aqueous layer		Volume of $\text{Na}_2\text{S}_2\text{O}_3$ used $V_a = (V_2 - V_1) \text{ cm}^3$
	Initial (V_1)	Final (V_2)	
5			
6			
7			
8			

Observation Table IV

Reagent Bottle Number	V_a (aqueous layer)	V_b (Organic layer)	$K_D = \left[\frac{V_b}{V_a} \right] \times 100$
1			
2			
3			
4			

Determination of K_D for I_2 in $\text{CCl}_4/\text{H}_2\text{O}$ 

1.3.5 Calculations

Estimation of the strength of sodium thiosulphate solution

Molarity of $K_2Cr_2O_7$ solution	$= M_1 = \dots\dots \text{mol dm}^{-3}$
Volume of $K_2Cr_2O_7$ solution	$= V_1 = \dots\dots 20\text{cm}^3$
Volume of $Na_2S_2O_3$ solution used	$= V_2 = \dots\dots \text{cm}^3$
(From Table I)	
Molarity of $Na_2S_2O_3$ solution	$= M_2 = ?$
Using the molarity equation ,	
$M_1 V_1 = 6 M_2 V_2$	
Molarity of $Na_2S_2O_3$ solution	$= M_2 = \frac{M_1 \times V_1}{6V_2}$
	$= \dots\dots \text{mol dm}^{-3}$

Preparation of 250 cm³ of M/500 $Na_2S_2O_3$ solution by dilution method for Aqueous layer:

$$\frac{1}{5} \times V_{Na_2S_2O_3} = \frac{1}{500} \times 250$$

$$V_{Na_2S_2O_3} = \frac{\frac{1}{500} \times 250}{\frac{1}{5}} = 2.5 \text{ cm}^3$$

2.5 cm³ of M/5 $Na_2S_2O_3$ should be added to 247.5cm³ of distilled water in a 250 cm³ volumetric flask.

Determination of K_D (Distribution Constant)

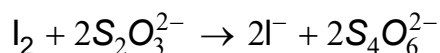
Formula used: $K_D = \frac{[c_{I_2}(\text{org.})]}{[c_{I_2}(\text{aq.})]}$

Reagent bottle Number	$K_D = \frac{[c_{I_2}(\text{org.})]}{[c_{I_2}(\text{aq.})]}$
1.	
2.	
3.	
4.	
	Mean $K_D = \underline{\hspace{2cm}}$

Calculation of Molarity of I_2 of Aqueous KI layer and Organic layer:

Formula used $M_{I_2} \times V_{I_2} = M_{Na_2S_2O_3} \times V_{Na_2S_2O_3}$

$$M_{I_2} = \frac{M_{Na_2S_2O_3} \times V_{Na_2S_2O_3}}{V_{I_2}}$$



1 mole 2 mole

1/2 mole 1 mole

Reagent Bottle Number	Concentration of free iodine in Organic layer $M_{I_2(org)} = \frac{M_{Na_2S_2O_3} \times V_{Na_2S_2O_3}}{V_{I_2}}$ $M_{I_2} = \frac{1/50 \times V_b}{5}$	Concentration of free iodine in Aqueous KI layer $c_{I_2(aq)} = \frac{K_D}{c_{I_2(org)}}$ $C_2 = \frac{K_D}{C_1}$
5		
6		
7		
8		

Estimation of strength (Molarity) of KI and KI_3 in Aqueous KI layer:

Reagent Bottle Number	Concentration of ($I_2 + KI_3$) in aqueous KI layer $M_{(I_2+KI_3)} = \frac{M_{Na_2S_2O_3} \times V_{Na_2S_2O_3}}{V_{(I_2+KI_3)}}$ $M_{(I_2+KI_3)} = \frac{0.005 \times V_a}{25}$ $C_3 = M_{(I_2+KI_3)}$	Concentration of KI_3 in aqueous KI layer = Concentration of ($I_2 + KI_3$) in aqueous KI layer – concentration of free iodine in aqueous KI layer $C_4 = C_3 - C_2$	Initial concentration of KI in aqueous KI layer $C_5 = \frac{0.1 \times V_{KI}}{90}$	Concentration of free KI in aqueous KI layer $C_6 = C_5 - C_4$
5				
6				
7				
8				

1.3.6 Result

The value of K_D (calculated) is found to be _____

Literature value of K_D is 85.52.

$$K_D = \frac{[C_{I_2} (org.)]}{[C_{I_2} (aq.)]}$$

(i) The distribution constant of iodine between CCl_4 and water is, $K_D =$

(ii) The equilibrium constant for the reaction, $\text{KI} + \text{I}_2 \rightarrow \text{KI}_3$ is, $K =$ ___ $\text{mol}^{-1} \text{dm}^3$.

Determination of Equilibrium Constant (K)

Knowing the equilibrium values of $[\text{I}_2]$, $[\text{KI}]$ and $[\text{KI}_3]$ for an aqueous layer, the equilibrium constant can be calculated.

$$[\text{I}_2] = C_2$$

$$[\text{KI}_3] = C_4$$

$$[\text{KI}] = C_6$$

$$\text{Equilibrium constant} = K = \frac{[\text{KI}_3]}{[\text{I}_2][\text{KI}]}$$

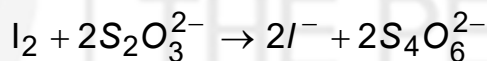
$$K = \frac{C_4}{C_2 \times C_6}$$

$$\text{Mean } K = \text{_____} \text{mol}^{-1} \text{dm}^3$$

Estimation of strength (Molarity) of I_2 of Aqueous KI layer and Organic layer:

Formula used, $M_{\text{I}_2} \times V_{\text{I}_2} = M_{\text{Na}_2\text{S}_2\text{O}_3} \times V_{\text{Na}_2\text{S}_2\text{O}_3}$

$$M_{\text{I}_2} = \frac{M_{\text{Na}_2\text{S}_2\text{O}_3} \times V_{\text{Na}_2\text{S}_2\text{O}_3}}{V_{\text{I}_2}}$$



1 mole 2 mole

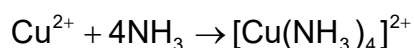
1/2 mole 1 mole

1.4 EXPERIMENT 1b: TO STUDY THE COMPLEX FORMATION BETWEEN COPPER IONS, Cu(II) AND AMMONIA AND DETERMINE THE FORMULA OF THE COMPLEX USING DISTRIBUTION TECHNIQUE

1.4.1 Principle

The distribution law can be used to study the complex formation between a solute and a solvent. The actual equilibrium concentrations of the various reactions can be determined.

Let us take the reaction between Cu(II) ions and NH_3 molecules which gives the deep blue coloured cuprammine complex.



... (1.12)

To study this complex, two immiscible solvents like water and CHCl_3 are taken. Distribution co-efficient, K_D , of ammonia between the two solvents is determined as

$$K_D = \frac{[\text{c}_{\text{NH}_3}(\text{org.})]}{[\text{c}_{\text{NH}_3}(\text{aq.})]} \quad \dots (1.13)$$

Next titration is done with standard HCl solution to find out the concentration of free ammonia, C_1 , and hence the number of moles n_1 , in the CHCl_3 layer. If the total number of moles of ammonia, n , added to the two layers initially is known, you can get the number of moles of ammonia, n_2 , in the aqueous layer as:

$$n_2 = n - n_1 \quad \dots (1.14)$$

After determining K_D , the next step is to determine the coordination number of NH_3 in the complex. This can be done by studying the distribution of NH_3 between a dilute aqueous Cu (II) solution and CHCl_3 layer. The concentration of free ammonia, C_3 , and hence the number of moles of NH_3 , n_4 , in the aqueous Cu(II) layer is thus the product of its distribution co-efficient and the number of moles of NH_3 in the CHCl_3 layer

$$K_D = \frac{n_{4(\text{aq})}}{n_{3(\text{org})}} \quad \dots (1.15)$$

$$n_4 = K_D \times n_3 \quad \dots (1.16)$$

As the total number of ammonia, N_T , added to the two layers initially is known, the number of moles of ammonia used for complex formation, n_{complex} can be determined as:

$$n_{\text{complex}} = N_T - (n_3 + n_4) \quad \dots (1.17)$$

The coordination number, the ratio x_i in the complex is obtained from the number of moles of ammonia used for complex formation to the number of moles of Cu(II) present and is given as:

$$x_i = \frac{n_{\text{complex}}}{\text{no. of moles of Cu(II)}}$$

1.4.2 Requirements

Apparatus

Stoppered bottles (200 cm^3) – 6

Dropper – 1

Burette (50 cm^3) – 1

Volumetric flask (250 cm^3) – 4

Conical flask (250 cm^3) – 1

Chemicals

Ammonia solution (molar solution),
 CHCl_3

Distilled H_2O

HCl (0.05 M HCl)

0.1 molar solutions of metal (Cu)
ions in the form of salts.

Methyl red (indicator)

Pipette (20 cm^3) – 1

Measuring cylinder (10 cm^3)-1

wash bottle for distilled water -1

Weighing bottle-1

Funnel (small) -1

Volumetric flask (1000 cm^3)-1

Burette stand -1

Caution: Liquor ammonia bottle should be opened very carefully away from eyes and never without prior cooling in ice. Even 1 M solution should not be measured with a pipette. It may cause mouth burns and lung irritation.

1.4.3 Procedure

Standardise the ammonia solution by diluting the 1 M stock solution ten times and titrating it against a standard HCl solution using methyl red as indicator.

1. Determination of distribution coefficient (K_D) of NH_3 between water and CHCl_3 .

- (a) Prepare 0.75 M and 0.5 M ammonia solution by dilution from this 1 M stock solution. Then prepare the following mixtures in three stoppered bottles.

Reagent bottle number	Concentration of $\text{NH}_3(\text{M})$	Volume of aqueous NH_3 taken (cm^3)	Volume of CHCl_3 (cm^3)
1	1.00	25	25
2	0.75	25	25
3	0.50	25	25

- (b) Stopper the bottles and shake vigorously for nearly half an hour and then allow the bottles to stand until contents of each bottle separate in two distinct layers.
- (c) Prepare 250 cm^3 of 0.1 M HCl solution.
- (d) **Titration of organic layer:** Pipette out 20 cm^3 of CHCl_3 layer and add about 200 cm^3 of water to it. Then add 5 drops of methyl red as indicator and titrate against the 0.5 M HCl solution till the end i.e. colour change from yellow to red and note the reading. From this reading, the concentration of free ammonia and hence the moles, n_1 , in the CHCl_3 layer is determined.
- (e) Thus, the number of moles, n_2 in the aqueous layer can be determined by subtracting the number of moles of NH_3 in CHCl_3 layer i.e. n_1 , from the total number of moles, n , added to the two layers initially.

- (f) By knowing the values of n_1 and n_2 , the distribution coefficient, K_D , for NH_3 in CHCl_3 and H_2O can be calculated.

$$K_D = \frac{n_{2(\text{aq})}}{n_{1(\text{org})}} \quad \dots (1.18)$$

Record the readings as in observation Table I, II and III.

2. Determination of Coordination number 'x'

- (a) Prepare the following mixtures in different reagent bottles.

Reagent bottle number	Volume of CHCl_3 (cm^3)	Volume of 1M aqueous NH_3 (cm^3)	Volume of CuSO_4 (cm^3)	Concentration of CuSO_4 (M)
4	75	25	25	0.1
5	75	25	25	0.08
6	75	25	25	0.06

- (b) Stopper the bottles and shake vigorously for nearly half an hour and then allow bottles to stand until contents of each bottle separate in two distinct layers.
- (c) Prepare 250 cm^3 of 0.1 M HCl solution.
- (d) **Titration of organic layer:** Pipette out 50 cm^3 of CHCl_3 layer and add about 150 cm^3 of water to it. Then add 5 drops of methyl red as indicator and titrate against the 0.5 M HCl solution till the end point i.e. colour change from yellow to red and note the reading. From this readings, the concentration of free NH_3 and hence the number of moles, n_3 , in the CHCl_3 layer is determined.
- (e) Thus, the number of moles of NH_3 , n_4 , in the aqueous Cu(II) layer is determined from the distribution coefficient, K_D and the number of moles of NH_3 in the CHCl_3 layer, n_3 .

$$K_D = \frac{n_{4(\text{aq})}}{n_{3(\text{org})}} \quad \dots (1.19)$$

$$n_4 = K_D \times n_3 \quad \dots (1.20)$$

- (f) Then calculate the total number of moles of ammonia, N_{NH_3} , added to the two layers initially. From this, the number of moles of ammonia used for complex formation, n_{complex} , can be determined

$$n_{\text{complex}} = N_{\text{NH}_3} - (n_3 + n_4) \quad \dots (1.21)$$

- (g) Then calculate the number of moles of Cu(II) ions. The ratio of the number of moles of ammonia used for complex formation to the number of moles of Cu(II) present gives the coordination number, x , in the complex.

Record the readings as in observation Table IV, V, VI and VII.

1.4.4 Observations

1. Preparation of 1.0 M NH₃ solution
2. Preparation of 0.75 M and 0.50 M NH₃ solution by diluting 1.0 M NH₃ solution.
3. Preparation of 0.1 M HCl solution.
4. Determination of K_D of NH₃ between H₂O and CHCl₃ layer

(a) Observation Table I

Reagent bottle number	Volume of CHCl ₃ (cm ³)	Concentration of NH ₃ , C(M)	Volume of aqueous NH ₃ , V (cm ³)	Total number of moles of NH ₃ $n = C(M) \times V(\text{cm}^3)$
1	25			
2	25			
3	25			

(b) Titration of 0.1 M HCl vs Organic CHCl₃ layer

Observation Table II

Reagent bottle number	Burette reading for organic layer		Volume of HCl used $V_{\text{HCl}} = (V_f - V_i) \text{ cm}^3$ $= V_1 \text{ cm}^3$
	Initial Reading (V_i)	Final Reading (V_f)	
1			
2			
3			

$$\text{Volume of HCl used} = (V_{\text{HCl}}) \text{ cm}^3 = V_1 \text{ cm}^3$$

Observation Table III

Reagent bottle number	Burette reading ($V_{\text{HCl}} = V_1 \text{ cm}^3$)	Concentration of NH ₃ in organic layer, $C_{\text{NH}_3} \times V_{\text{NH}_3} =$ $C_{\text{HCl}} \times V_{\text{HCl}}$ $C_{\text{NH}_3} = \frac{0.1 \times (V_1)}{20} \text{ (M)}$	Number of moles of NH ₃ in organic layer = (Concentration of NH ₃ in organic layer) x (Volume of organic CHCl ₃ taken in mixture) $n_1 = C_{\text{NH}_3} \times V$ $n_1 = C_{\text{NH}_3} \times \frac{25}{1000}$	Total number of moles of NH ₃ (n)	Number of moles of NH ₃ in aqueous layer $n_2 = n - n_1$	Distribution coefficient $K_D = \frac{n_2}{n_1}$
1						
2						
3						

Mean $K_D = \underline{\hspace{2cm}}$

(a) Observation Table IV

Reagent bottle number	Volume of CHCl_3 (cm^3)	Volume of 1M aqueous NH_3 (cm^3)	Total number of moles of NH_3 $M_{\text{NH}_3} = c_{\text{NH}_3} \times V_{\text{NH}_3}$
4	75	25	
5	75	25	
6	75	25	

(b) Observation Table V

Reagent bottle number	Concentration of CuSO_4 , $c_{\text{Cu}^{2+}}$ (M)	Volume of CuSO_4 , $V_{\text{Cu}^{2+}}$ (cm^3)	Number of moles of CuSO_4 , $n_{\text{Cu}^{2+}} \times V_{\text{Cu}^{2+}}$
4	0.1	25	
5	0.08	25	
6	0.06	25	

(c) Titration of 0.1 M HCl vs Organic layer

Observation Table VI

Reagent bottle number	Burette reading for organic layer		Volume of HCl used $V_{\text{HCl}} = (V_f - V_i) \text{cm}^3$ $= V_2 \text{cm}^3$
	Initial (V_i)	Final (V_f)	
5			
6			
7			

Volume of HCl used = $V_{\text{HCl}} = V_2 \text{cm}^3$

(d) Observation Table VII

	Burette reading ($V_{\text{HCl}} = V_2(\text{cm}^3)$)	Concentration of NH_3 in organic layer, $c_{\text{NH}_3}(\text{org.})$ $c_{\text{NH}_3}(\text{org.}) \times V_{\text{NH}_3}(\text{org.}) = c_{\text{HCl}} \times V_{\text{HCl}}$ $c_{\text{NH}_3}(\text{org.}) = \frac{0.1 \times (V_1)}{50}$	Number of moles of NH_3 organic layer, (Concentration of NH_3 in organic layer) $\times$ (Volume of organic CHCl_3 taken in mixture) $n_3 = c_{\text{NH}_3} \times V$ $= \frac{0.1 \times (V_2)}{50} \times \frac{75}{1000}$	Total number of moles of NH_3 in aqueous layer $n_4 = K_D \times n_3$	Number of moles of NH_3 used for complex formation $n_{\text{complex}} = M_{\text{NH}_3} - (n_3 - n_4)$	Coordination number, $x = \frac{n_{\text{complex}}}{n_{\text{Cu}^{2+}}}$
1						
2						
3						

Mean 'X' _____

1.4.5 Calculations

Calculation of coordination number 'x'

1.4.6 Result

The coordination number of copper in the copper-ammonia complex, determined using chloroform as non-aqueous solvent is _____ (Literature value = 4)

Thus, the formula of the complex using distribution technique is $[\text{Cu}(\text{NH}_3)_4]^{2+}$

1.5 ANSWERS TO SAQs

1. Section 1.2.2
2. Eq. 1.3



EXPERIMENT 2

CONSTRUCTION OF THE PHASE DIAGRAM OF A SIMPLE EUTECTIC SYSTEM USING COOLING CURVES METHOD

Structure

2.1	Introduction	2.4	Procedure
	Expected Learning Outcomes	2.5	Observations
2.2	Principle	2.6	Result
2.3	Requirements		

2.1 INTRODUCTION

In the previous experiment you have learnt about the complexation equilibrium using distribution method. In the present experiment you would study solid-liquid heterogeneous equilibrium in a binary system. In this, you would learn about the construction of phase diagram for a simple eutectic system using thermal analysis (or cooling curve) method. You would study the solid-liquid equilibria by taking naphthalene-benzoic acid system as an example for the simple eutectic system and construct a phase diagram for the same. The study of solid-liquid equilibria is quite important as it provides information about the processes related to alloy formation and crystallisation (purification) etc.

In the next experiment you would study about the determination of the critical solution temperature (CST) of phenol-water system.

Expected Learning Outcomes

After studying about and performing this experiment, you should be able to

- ❖ define a simple eutectic system and state its importance;
- ❖ explain the thermal analysis(or cooling curve) method of studying the phase equilibria in two-component systems;

- ❖ define a cooling curve and give the significance of break and halt observed in it;
- ❖ record the temperature-time data for cooling of melts of mixtures of naphthalene and benzoic acid having different compositions;
- ❖ plot the cooling curves for the mixtures of different compositions of naphthalene-benzoic acid system using temperature-time data;
- ❖ construct the phase diagram for the naphthalene-benzoic acid system using the data from cooling curves; and
- ❖ identify the eutectic point in the phase diagram for naphthalene-benzoic acid system and report the eutectic temperature and composition for the same.

2.2 PRINCIPLE

A phase diagram is a graphical representation of the nature of equilibrium in a heterogeneous system under given set of conditions. For a one-component system it represents the number of phases that exist at a given set of values of pressure and temperature for a pure substance. On the other hand, for a two-component system displaying solid-liquid equilibrium, it represents the nature and compositions of phases in equilibrium under given set of temperature and composition conditions.

Naphthalene and benzoic acid represent a heterogeneous system in which the components are completely miscible in the liquid state and are completely immiscible in the solid state. This system is an example of a simple-eutectic system in which the mixture of constituents having a certain fixed composition melts (and freezes) at a fixed temperature. This temperature is lower than the melting points of the individual constituents or their mixture having any other composition. Such a composition is called **eutectic composition** and the corresponding temperature is termed **eutectic temperature**. In such a case (i.e., for eutectic composition) the overall composition of the solid and liquid phase is identical at equilibrium. A schematic representation of a typical T - c phase diagram for a simple eutectic system (A-B) is given in Fig. 2.1. Please refer Unit 4 of BCHCT-135 course for its explanation.

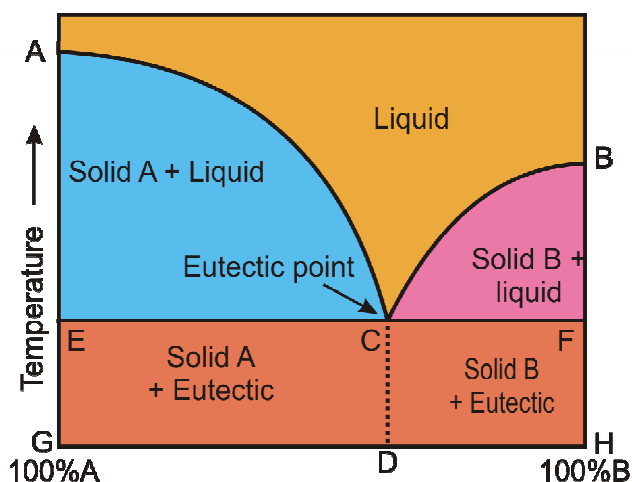


Fig. 2.1: A schematic representation of the phase diagram of a simple eutectic system (AB).

In order to construct the phase diagram for a simple eutectic system we need to know about the nature of phases in equilibrium at different temperatures and the transition temperatures for different sets of compositions of the two components. As the phase transitions in such systems are accompanied by significant enthalpy changes these can be easily followed by thermal analysis or **cooling curves method**. In this method, the mixtures of two components with different compositions are heated to melt and then allowed to gradually cool down. The temperature of the mixture is followed as a function of time. The observed data is then plotted as cooling curves showing the variation of temperature with time and the temperatures corresponding to different phase changes (transitions). These cooling curves are then used to construct the phase diagram by plotting these transition temperatures against the corresponding composition of the mixtures.

To construct the phase diagram for naphthalene-benzoic acid system, we will prepare different mixtures of varying composition of naphthalene and benzoic acid ranging from pure naphthalene to pure benzoic acid. These mixtures will then be heated one by one to obtain the corresponding melts. These melts will then be allowed to cool gradually and the temperature of the system will be recorded as a function of time. The data so obtained will then be plotted as a cooling curves i.e., the graphs between temperature and time for each of the studied compositions. A schematic representation of set of typical cooling curves for different compositions of a two component (A-B) system are given in Fig. 2.2 a).

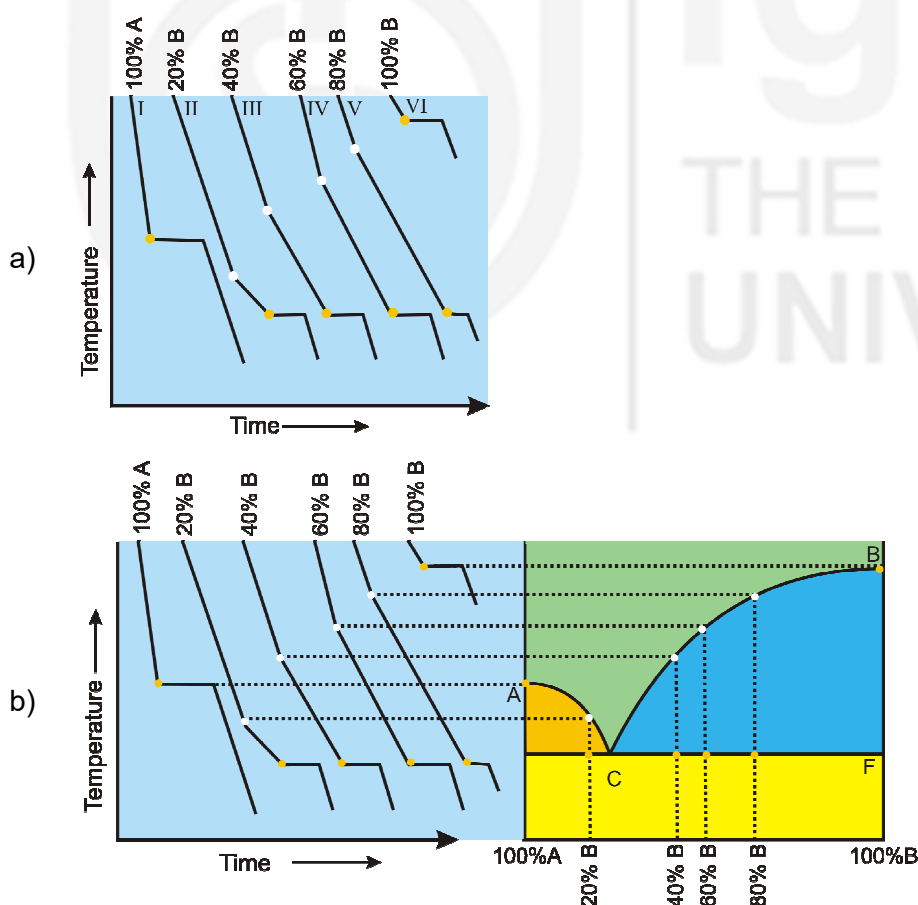


Fig. 2.2: a) Schematic representation of the cooling curves of pure components of a two component (A-B) system and their mixtures b) constructing the phase diagram from cooling curves.

The phase transitions in different mixtures are recorded in terms of temperatures corresponding to the change in the slope of cooling curves (called **breaks**) and constant temperature regions (called **halts**). The breaks in a cooling curve correspond to the separation of the solid (that is in excess) from the melt. These arise due to the heat evolved during the crystallisation of the solid from the melt. At this stage, a solid phase and the melt are in equilibrium and according to the phase rule for condensed systems the system is **univariant**. At low mass percentages of benzoic acid, it will be naphthalene that will separate first whereas, in mixtures having higher mass percentages of benzoic acid; the benzoic acid would separate first.

The halt in the cooling curve arises when the system reaches a certain characteristic temperature called **eutectic temperature**, where both the components start crystallising out and the temperature becomes constant. The system at this stage has three phases in equilibrium: two solids and a liquid or melt. Therefore, according to the phase rule for condensed systems, the system becomes **invariant**. You should note that in the cooling curves for different mixtures the halt will be observed at the same temperature irrespective of their composition because the composition of the solid phase that separates out (i.e., eutectic mixture) is same in all the cases. The temperatures at which the break and halt are observed for a given composition are then plotted against the same composition in the temperature-composition plot to construct the phase diagram. Construction of phase diagram for a system (A-B) from the cooling curves of mixtures of varying composition of A and B is shown schematically in Fig. 2.2 b).

2.3 REQUIREMENTS

Apparatus		Chemicals
Boiling tube	1	Benzoic acid
Test tubes	12	Naphthalene
Rubber corks(for test tubes)	1	Liquid paraffin
Rubber cork(for boiling tube)	1	
Thermometer 360°	1	
Stirrer	1	
Beaker (250 cm ³) or paraffin bath	1	
Iron clamp and stand	1	
Bunsen burner	1	
Wire gauze	1	
Tripod stand	1	
Stopwatch	1	

2.4 PROCEDURE

There are following four parts of the experiment.

- A. Preparing mixtures of naphthalene and benzoic acid having varying composition
- B. Obtaining temperature-time data for the cooling of melts of mixtures of different compositions
- C. Drawing cooling curves from temperature-time data
- D. Constructing phase diagram from the cooling curves

Let us learn about the procedure for these steps:

A. Preparing mixtures of naphthalene and benzoic acid having varying composition

1. Take eleven test tubes and label them from 1 to 11. Alternatively, you may label them as per the composition given in the fourth row of the following table.

S.No.	1	2	3	4	5	6	7	8	9	10	11
Mass of Naphthalene /g	10	9	8	7	6	5	4	3	2	1	0
Mass of benzoic acid/ g	0	1	2	3	4	5	6	7	8	9	10
Composition / (m/m % of Naphthalene)	100	90	80	70	60	50	40	30	20	10	0

2. Weigh naphthalene and benzoic acids in the labeled test tubes as per the details given in the table. (You may be provided with the prepared mixtures of these compositions. In such a case just weigh about 10 g each of the sample in the test tube labeled for the corresponding composition)

B. Obtaining temperature-time data for the cooling of melts of mixtures of different compositions

Follow the instructions given as under and record your observations in the Observation Table 2.1 given below (Section 2.5)

1. Take the first test tube, labeled as 1 and keep it in liquid paraffin bath (this bath can be prepared by pouring liquid paraffin in the beaker and keeping the beaker on a wire gauze kept on the tripod stand over the Bunsen burner)
2. Heat the test tube, and when all the solid has melted take out the test tube; wipe to clean it and fix the thermometer and the stirrer into it through the rubber cork.
3. Insert this test tube into the boiling tube through a rubber cork and fix it as shown in the Fig. 2.3. Mount this assembly on an iron stand.

The boiling tube provides an air jacket and allows the cooling to be gradual.

4. Record the temperature of the melt and start the stopwatch. Keep stirring the mixture slowly and record the temperature every half a minute till the melt is completely solidified. Record your data in Observation Table-2.1.
5. Repeat step 1-4 for all the eleven mixtures of different compositions and record your data in Observation Table-2.1.

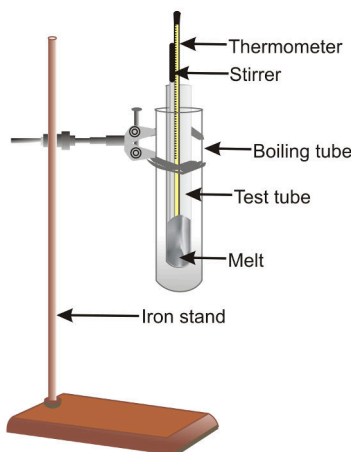


Fig. 2.3: Experimental set up for studying naphthalene-benzoic acid system by cooling curve method.

C. Drawing cooling curves from temperature-time data

1. Use the data from Observation Table 2.1 to plot a graph between the temperature (on y-axis) and time (on x-axis) for all the compositions studied. You may plot the graphs on the graph 2.1 given in Section 2.5.

D. Constructing phase diagram

Follow the instructions given as under and record your observations in the Observation Table 2.2 given below (Section 2.5)

1. From the plots of cooling curves for all the compositions, note down the temperature for the initial solidification (break in the curve) and the temperature when both the solids are separated (halt in the curve) in Observation Table 2.2.
2. Using the data from Observation Table 2.2, mark the break points and the halts corresponding to all the studied compositions on the graph 2.2 provided in Section 2.5. The compositions are to be plotted on the x-axis whereas the corresponding temperatures for the breaks and halts are plotted on the y-axis.
3. Join the break points and halts as per the Fig. 2.2 b) given above to get the phase diagram for naphthalene-benzoic acid system.

2.5 OBSERVATIONS

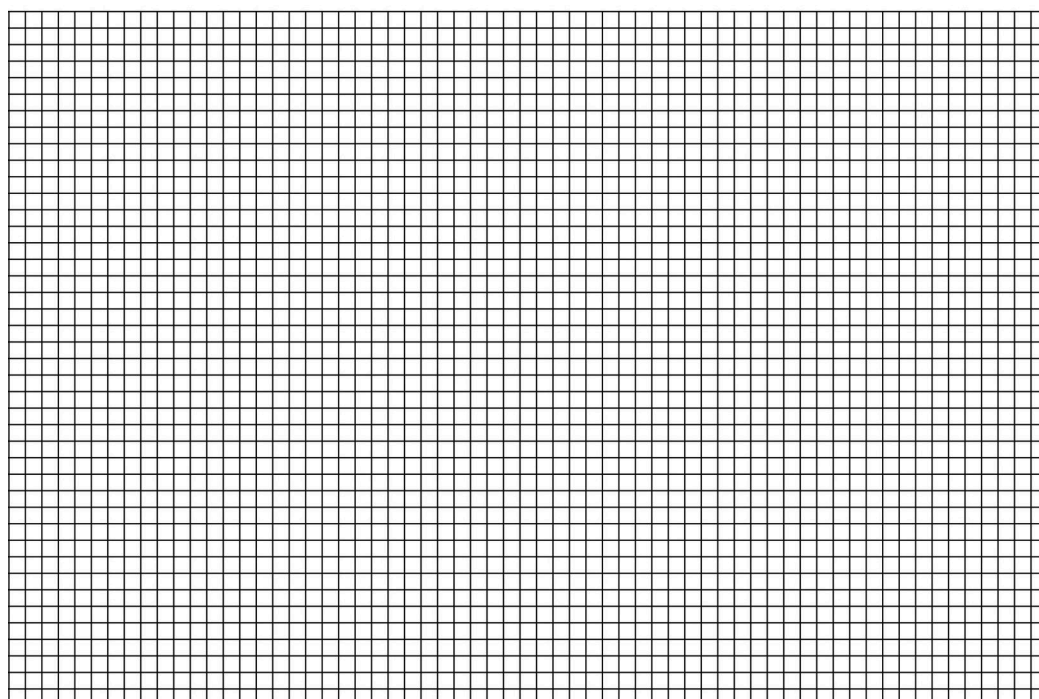
Record your observations on temperature-time data for different compositions in the table given below:

Observation Table-2.1: Temperature-time data for mixtures of different compositions

Mix. No. Time /min	1	2	3	4	5	6	7	8	9	10	11
0.5											
1.0											
1.5											
2.0											
2.5											
3.0											
3.5											
4.0											
4.5											
5.0											
5.5											
6.0											
6.5											
7.0											
7.5											
8.0											

Graph 2.1

Draw the cooling curves for different compositions of naphthalene-benzoic acid system here (Use an extra sheet of graph paper, if required)



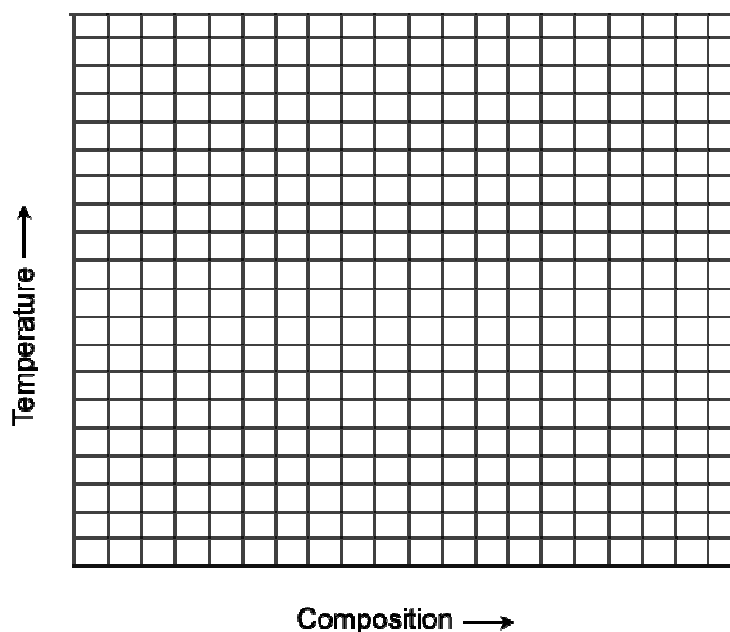
Observation Table-2.2: Summary of the temperature at the break points and halts in the cooling curves of mixtures of different compositions

S. No of the mixture	Composition Mass % of naphthalene	Temperature at the break in cooling curve / °C	Temperature at the halt in the cooling curve / °C
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			

Constructing phase diagram

Use the data from Observation Table 2.2 and mark the temperatures for breaks and the halts (on y-axis) for different compositions (x-axis) to construct the phase diagram.

Graph 2.2



2.6 RESULT

- The phase diagram for the simple eutectic system (naphthalene and benzene) has been obtained and the eutectic temperature has been observed to be°C.
- The composition at the eutectic point = ... m/m % (naphthalene)

EXPERIMENT 3

STUDY OF THE VARIATION OF MUTUAL SOLUBILITY TEMPERATURE WITH CONCENTRATION FOR THE PHENOL- WATER SYSTEM AND DETERMINATION OF CRITICAL SOLUTION TEMPERATURE

Structure

3.1	Introduction	3.5	Observations
	Expected Learning Outcomes	3.6	Calculations
3.2	Principle	3.7	Results
3.3	Requirements	3.8	Terminal Questions
3.4	Procedure	3.9	Answers

3.1 INTRODUCTION

In Unit 2 of the BCHCT-135 course, you have studied about *critical solution temperature*(CST)of two partially miscible liquids and *the distribution law*.

You have already performed two experiments, i.e. Experiments 1 and 2 based on the distribution law. This experiment and Experiment 4 are related to the critical solution temperature. In this experiment, we will study of the variation of mutual solubility temperature with concentration for the phenol- water system and determine of critical solution temperature

After performing this experiment, you should be able to:

Expected Learning Outcomes

After performing this experiment, you should be able to:

- ❖ define critical solution temperature;
- ❖ give examples of partially miscible liquid pairs having upper, lower and upper as well as lower critical solution temperatures; and
- ❖ determine the CST of partially miscible liquid pairs such as phenol and water.

3.2 PRINCIPLE

In Unit 2 of the BCHCT-135 course, you studied that some liquid pairs do not form solutions in all compositions. Such liquid pairs are said to be *partially miscible liquids*. However, due to increased solubility with increase or decrease of temperature, these may become completely miscible.

We can understand such a system by using the example of a system containing phenol and water. When a very small amount of phenol is added to water at room temperature, it dissolves completely to give a *single liquid phase*. However, when the addition of phenol is continued, a point is reached when phenol does not dissolve anymore. At this point, *two phases*, i.e., two liquid layers are formed—one consisting of water saturated with phenol and the other containing phenol saturated with water. Further addition of phenol causes water to shift from water-rich layer to phenol-rich layer.

If the addition of phenol is continued, a point is reached when phenol acts as a solvent for all the water present and the two phases merge with each other to form a single phase, i.e., solution of water in phenol. Thus, on shaking equal volumes of phenol and water, two layers are formed—one of saturated solution phenol in water and the other of saturated solution water in phenol.

It has been experimentally found that at a constant temperature, the composition of the two layers, although different from each other, remains constant as long as the two phases are present. Such *solutions of different compositions coexisting with each other* are termed as **conjugate solutions**.

The addition of small amounts of phenol or water changes the volumes of the two layers and not their compositions. As the temperature is increased, the behaviour remains the same except that the mutual solubility of the two increases. When the temperature reaches 338.8K, the composition of the two layers becomes identical and thereafter, the two liquids are completely miscible; i.e., at and above 338.8K, phenol and water dissolve in each other in all proportions and yield only a single liquid layer on mixing.

The variation of mutual solubility of water and phenol with temperature is shown in Fig. 3.1

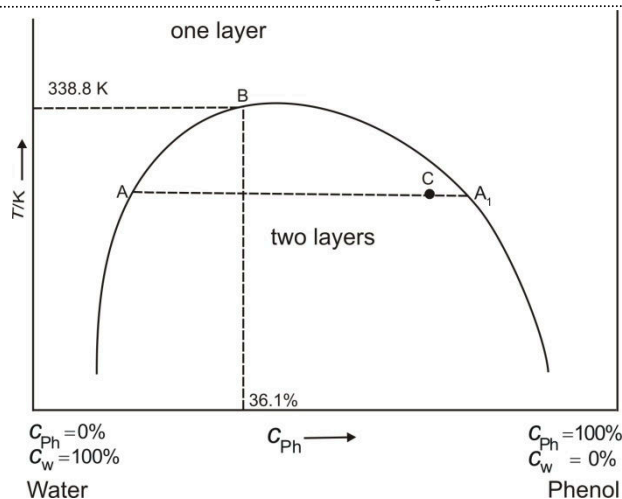


Fig. 3.1: Phenol-water system; C_{Ph} stands for composition of phenol and c_w for composition of water.

At a particular temperature, say 325 K point A represents the composition of water-rich layer and point A_1 , represents the composition of phenol-rich layer in equilibrium with the first layer. Between these two compositions, all mixtures will yield two layers of compositions A and A_1 . Outside these compositions, the two liquids are soluble mutually at 325 K. Similar behaviour is seen at other temperatures below 338.8 K. We can conclude that the dome-shaped area represents the range of existence of two liquid phases and the area outside the dome represents a single liquid phase. The temperature corresponding to the point B, i.e., the temperature at which the solubility becomes complete is called the **critical solution temperature** or the **consolute temperature**.

Since the *mutual solubility of phenol and water increases with rise in temperature*, the critical solution temperature (CST) lies on top of the dome. Hence, such liquid systems are said to possess an *upper critical solution temperature* or *upper consolute temperature*. Thus, the critical solution temperature, for phenol-water system is 338.8 K. At and above 338.8K, phenol and water are completely miscible with each other in all proportions. At this temperature, the composition of the solution is 36.1% phenol and 63.9% water.

Here, you can consider the line formed by joining the points A and A_1 as the *tie line* and consider a point C on it. Similar to the interpretation of lever rule for liquid–vapour equilibrium studied in Unit 1, here also we can use lever rule as the two layers are in equilibrium. Thus, at any point C, the relative amounts of the two separate layers are given by the relationship,

$$\frac{\text{Mass of the layer A}}{\text{Mass of the layer } A_1} = \frac{A_1C}{AC} \quad \dots (3.1)$$

There are some liquid pairs (e.g., triethylamine-water) for which *mutual solubilities decrease with rise in temperature*. As the temperature is decreased, the mutual solubilities increase and *below the consolute temperature*, the two liquids become miscible in all proportions. Such systems possess *lower consolute temperature*. The variation of mutual solubility of triethylamine and water with temperature is shown in Fig. 3.2.

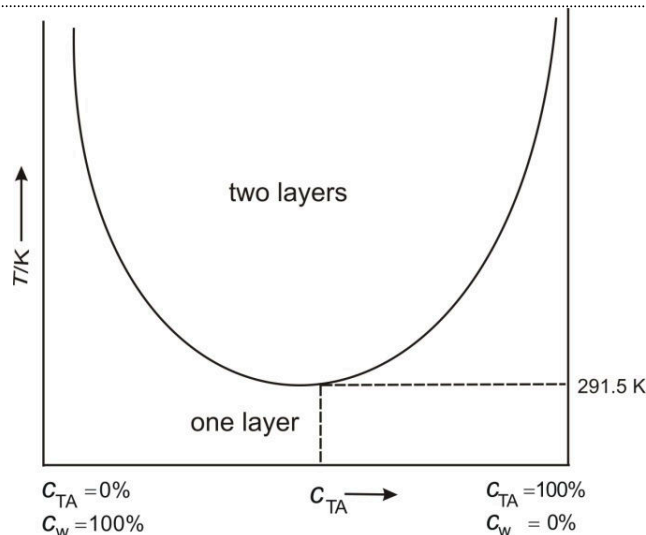


Fig. 3.2: Triethylamine-water system; C_{TA} stands for the composition of triethylamine.

Above 291.5K, on shaking triethylamine and water two layers are formed; but below 291.5 K, triethylamine and water are completely miscible with each other in all proportions.

There are a few liquids pairs, e.g., nicotine and water which show *both* the upper and lower consolute temperatures. These liquid pairs are completely miscible above a certain temperature (upper consolute temperature) and also below a certain temperature (lower consolute temperature). The variation of mutual solubilities of nicotine and water with temperature is shown in Fig. 3.3.

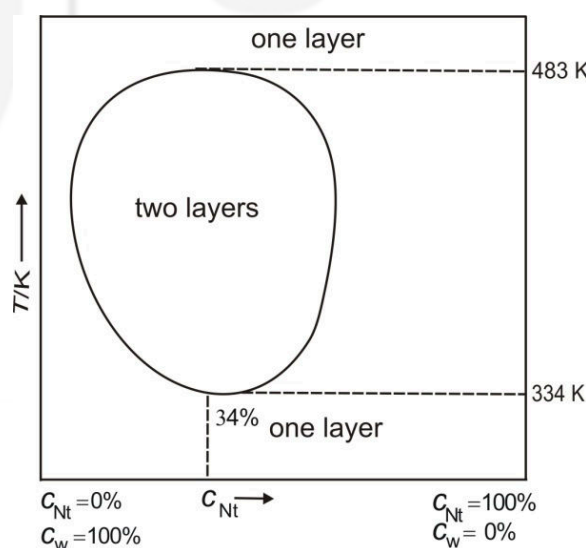


Fig. 3.3: Nicotine-water system; C_{Nt} stands for the composition of nicotine.

Within the enclosed area, the two liquids are only partially miscible, while outside the enclosed area, they are completely miscible. The composition corresponding to both the upper and the lower consolute temperatures is the same, viz., 34% nicotine.

Table 3.1 lists the consolute temperatures of some liquid pairs. The two liquid components are denoted as A and B.

Table 3.1: Consolute Temperatures of Some Liquid Pairs

Components		Consolute temperature / K	
A	B	Upper	Lower
Water	Phenol	338.8	—
Aniline	Hexane	333.6	—
Methanol	Carbon disulphide	323.5	—
Water	Diethylamine	—	316
Water	Triethylamine	—	291.5
Water	Nicotine	483	334
Water	<i>m</i> -Toluidine	393	280

SAQ 1

Fill in the blanks with appropriate word:

- The two liquid layers in equilibrium are known as -----solutions.
- The temperature at which the two partially miscible liquids first become miscible in all proportions is called -----temperature.
- The critical solution temperature is also called----- temperature.

Now you know that phenol and water constitute a partially miscible liquid pair which has an upper CST. The objective of this experiment is to determine the CST of this system.

In this experiment, we will determine miscibility temperatures of different mixtures of phenol and water. A graph is plotted by taking percentage composition of mixture along x-axis and miscibility temperature along the y-axis. The temperature corresponding to the highest point on the dome shaped curve gives the CST of the system.

3.3 REQUIREMENTS

Apparatus

- | | | |
|---|--------------------------------------|--------|
| 1 | Beaker 250 cm ³ | 1 No. |
| 2 | Boiling tubes | 5 No. |
| 3 | Burettes | 2 Nos. |
| 4 | Burner | 1 No. |
| 5 | Corks | 1 No. |
| 6 | Graduated pipette 10 cm ³ | 1 No. |

7	Thermometer 100°	1 No.
8	Wire gauze	1 No.
9	Wire stirrer	1 No

The stock solution can be prepared by adding 20 g of distilled water to every 80 g of phenol.

Stock Solution Provided

1. Phenol 80% (mass/mass)

3.4 PROCEDURE

1. Set up two burettes, one for phenol solution and the other for distilled water. Take five boiling tubes and number them from 1 to 5.
2. Then, prepare the following mixtures of phenol and water in test tubes:

Boiling tube no.	1	2	3	4	5
80% Phenol Soln. (cm ³)	10.0	9.0	8.0	7.0	6.0
Water (cm ³)	0.0	1.0	2.0	3.0	4.0
% Phenol (mass/mass)	80	72	64	56	48

3. Take solution no. 1 in the boiling tube.
4. Fix the wire stirrer and thermometer in the boiling tube through the cork and place the boiling tube in the water bath. The bulb of the thermometer should dip in the phenol-water mixture. The level of water in the bath should be 1 cm above the level of mixture in the boiling tube, see (Fig. 3.4).

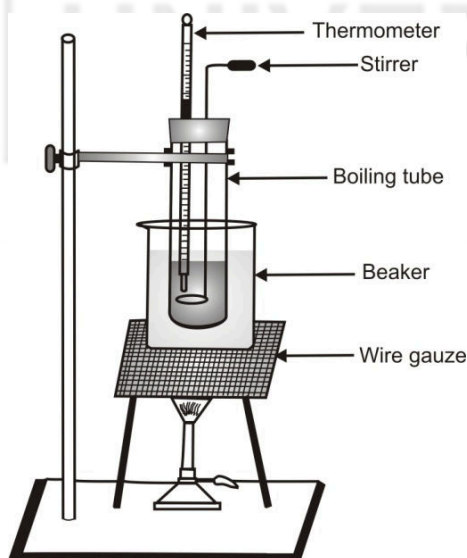


Fig 3.4: Determination of miscibility temperature.

5. The phenol-water mixture in the boiling tube may give rise to two layers. Stir the mixture with the wire stirrer. It will produce a cloudiness or turbidity in the tube.

6. Raise the temperature of the bath slowly while stirring the mixture constantly. *Note down the temperature (in Table 3.2) of the bath at which the last trace of cloudiness disappears.*
7. Remove the burner and allow the clear solution to cool slowly while keeping it stirred. *Note down the temperature at which cloudiness just reappears.*
8. The average of the two temperatures, which should be nearly equal, is the **miscibility temperature** for the mixture. Transfer the mixture into test tube no. 1.

If the phenol-water mixture does not produce two layers, it means that the miscibility temperature of this mixture is below room temperature. Cool the mixture with ice-cold water. Keep on stirring the mixture. Note down the temperature at which cloudiness just appears in the mixture. Cool the mixture to 4 & 5° below this temperature and then remove the cold bath. Allow the mixture to warm naturally and note down the temperature at which cloudiness just disappears. Take average of the two temperatures, which would give the **miscibility temperature** of this mixture.

9. Find out the miscibility temperature for each mixture kept in the boiling tubes 1 to 5 in the same manner and record the values in Table 3.2.
10. Now dilute the mixtures kept in the boiling tubes 1 to 5 with an equal volume of water (i.e., 10 cm³). This will give five more mixtures (no. 6-10) having 40,36,32,28 and 24% phenol, respectively.
11. Find miscibility temperatures for these also and record the values in Table 3.2.

After the experiment, transfer all the mixtures to a **used phenol** bottle kept in the laboratory for recovery of phenol by fractional distillation.

3.5 OBSERVATIONS

Room temperature = ...-°C

Table 3.2::% Composition - Miscibility Temperature Data

Sl.No.	%Phenol	Miscibility temperature / °C		
		Turbidity disappears	Turbidity re-appears	Average
1	80			
2	72			
3	64			
4	56			
5	48			
6	40			

7	36			
8	32			
9	28			
10	24			

3.6 CALCULATIONS

Plot a graph by taking percentage composition of mixture along the x-axis and the miscibility temperature along the y-axis. Draw a smooth curve through the points. Mark the maximum point on the curve. Read the temperature and the composition of the mixture at the maximum point from the graph. The temperature for the maximum point on the graph is the critical solution temperature for the phenol-water system.

3.7 RESULT

The critical solution temperature (CST) of phenol-water system = ...°C

Precautions

1. Phenol should be handled with abundant care. Phenol is a highly poisonous and strongly caustic substance. It produces painful burns on coming in contact with skin.
2. In case of accidental contact with phenol or its solution, wash the affected part with plenty of water and then apply Vaseline or skin cream.

3.8 TERMINAL QUESTIONS

1. i) A system having lower critical solution temperature is-----
 ii) A system having both the upper and the lower critical solution temperatures is----- .

3.9 ANSWERS

Self Assessment Questions

1. i) conjugate
 ii) critical solution or consolute
 iii) consolute

Terminal Questions

1. i) triethylamine-water
 ii) nicotine-water.

EXPERIMENT 4

STUDY OF THE EFFECT OF IMPURITIES ON CST OF PHENOL-WATER SYSTEM

Structure

4.1	Introduction	4.5	Calculations
	Expected Learning Outcomes	4.6	Observations
4.2	Principle	4.7	Result
4.3	Requirements	4.8	Terminal Questions
4.4	Procedure	4.9	Answers

4.1 INTRODUCTION

In the previous experiment, you have learnt how to determine the critical solution temperature of phenol-water system.

You also studied in Unit 2 of the BCHCT-135 course that the presence of small amounts of impurity produces a very large change in critical solution temperature and the change in CST values is usually a linear function of concentration of impurity. Thus, the amount of impurity present in a system can be determined by measuring the CST values.

In this experiment, you will study the effect of an impurity (sodium chloride) on the critical solution temperature (CST) of the phenol-water system. You will also determine the concentration of NaCl in an unknown solution by critical solution temperature method.

Expected Learning Outcomes

After performing the given experiments you should be able to:

- ❖ describe the effect of impurity on critical solution temperature; and
- ❖ determine the concentration of NaCl in a solution by the CST method;

4.2 PRINCIPLE

You studied in Unit 2 of the BCHCT-135 course studied that the impurities have a marked effect on the CST of a partially miscible liquid pair.

The presence of an impurity, dissolved in one or both of the phases, changes the CST values as well as the liquid phase composition at CST. Substances soluble in only one of the liquids raise the upper CST and lower the lower CST.

Substances which are soluble in both the liquids tend to lower the upper CST and raise the lower CST.

Sodium chloride is soluble only in water and not in phenol. Therefore, it will raise the CST of phenol-water system. Here, we will test this concept by performing this experiment.

In this experiment, the miscibility temperatures for various mixtures of phenol and aqueous solutions of sodium chloride will be determined. A calibration curve (usually a straight line) is plotted between the concentration of NaCl and the miscibility temperature.

The miscibility temperature for a mixture of phenol and the NaCl solution unknown concentration is also determined. From the calibration curve, the concentration of NaCl solution can then obtained.

Now answer the following SAQs before performing the experiment.

SAQ 1

What is the effect of impurity on CST? Explain.

SAQ 2

How will the CST of a system change, if the impurity dissolves in

- i) one of the liquids, and
 - ii) both the liquids?
-

4.3 REQUIREMENTS

Apparatus

Beaker 250 cm ³	1 No;
Boiling tubes	7 No.
Burettes	2 Nos.
Burner	1 No.
Cork	1 No.
Graduated pipette	1 No.

Thermometer, 100°	1 No.
Wire stirrer	1 No.
Wire gauze	1 No.

Stock Solution Provided

1. Phenol 80% (mass/mass)
2. Sodium chloride 1% (mass/volume)

Phenol solution can be prepared as described in Experiment 3. Sodium chloride solution can be prepared by dissolving 1 g of NaCl in every 100 cm³ of solution.

4.4 PROCEDURE

1. Take seven boiling tubes and number them from one to seven. Add 5 cm³ of 80% (mass/mass) phenol solution to each boiling tube with the help of a burette.
2. From another burette, add 1,2,3,4 and 5 cm³ of 1% NaCl solution to boiling tubes numbered 2 to 6, respectively.
3. Add 5 cm³ of NaCl solution of unknown concentration to the boiling tube numbered 7.
4. Now, add 5,4,3,2 and 1 cm³ of distilled water to boiling tubes numbered 1 to 5, respectively. This makes the volume of mixture 10 cm³ in each boiling tube.
5. Find out miscibility temperatures of each mixture in the boiling tubes 1 to 7 as described in Experiment 3. Record the data in Table 4.1.

4.5 OBSERVATIONS**Table 4.1: Composition-Miscibility Temperature Data**

Boiling Tube No.	(Volume of phenol soln. taken)/cm ³	(Volume of 1%NaCl taken)/cm ³	(Volume of distilled water taken)/cm ³	% NaCl in aqueous solution	Miscibility Temperature/°C		
					Turbidity disappears	Turbidity reappears	Mean
1	5	0.0	5.0	0.0			
2	5	1.0	4.0	0.20			
3	5	2.0	3.0	0.40			
4	5	3.0	2.0	0.60			
5	5	4.0	1.0	0.80			
6	5	5.0	0.0	1.00			
7	5	5.0	0.0	Unknown			

4.6 CALCULATIONS

Draw a graph between the percentage of sodium chloride (taken at x-axis) and the miscibility temperature (taken at y-axis). Find out the percentage of sodium

chloride in the solution of unknown concentration (from the graph) corresponding to its miscibility temperature, using interpolation method.

4.7 RESULT

Sodium chloride is soluble only in water and not in phenol. Therefore, it has raised the CST of phenol-water system.

Concentration of NaCl in unknown solution = ... %.

Precautions

1. Phenol should be handled with abundant care. Phenol is a highly poisonous and strongly caustic substance. It produces painful burns on coming in contact with skin.
2. In case of accidental contact with phenol or its solution, wash the affected part with plenty of water and then apply vaseline.

4.8 TERMINAL QUESTIONS

1. How will the CST of phenol-water system change when the following are added to it?
 - i) Naphthalene (insoluble in water but soluble in phenol)
 - ii) KCl (soluble in water but insoluble in phenol)
 - iii) Sodium oleate (soluble in both water and phenol)
2. In this experiment, how has the CST of phenol-water system changed with the change in the concentration of the impurity i.e., NaCl? Can you explain the trends observed by you?

4.9 ANSWERS

Self Assessment Questions

1. Impurities have a marked effect on CST. The presence of an impurity, dissolved in one or both of the phases, changes the CST values as well as the liquid phase composition at CST.
2. If an impurity is soluble only in one of the two liquids, it will raise the upper CST, while the lower CST will be lowered. If on the other hand, it is soluble in both the liquids, then upper CST will be lowered, while the lower CST will be raised.

Terminal Questions

1.
 - i) Upper CST will increase.
 - ii) Upper CST will increase
 - iii) Upper CST will decrease and lower CST will increase.
2. The actual observed trends in the experiment in the variation may be stated and explained.

EXPERIMENT 5

DETERMINATION OF DISSOCIATION CONSTANT OF A WEAK ACID USING A pH-METER

Structure

5.1	Introduction	5.5	Observations
	Expected Learning Outcomes	5.6	Calculations
5.2	Principle	5.7	Result
5.3	Requirements	5.8	Answers to SAQ's
5.4	Procedures		

5.1 INTRODUCTION

In the previous practical courses (BCHCT 132L), we have discussed different types of quantitative analysis. Also acid base titration was defined there. Here a detailed description of acid-base titrations is done to help you understand and perform the experiment in which you will determine the dissociation constant of a weak acid using a meter. Please refer to Unit 1 of BCHCL 132 where introduction to Titrimetry has already been done. Acid-base titration is a quick and accurate method of determining acidic or basic substances in analytical samples. A standard solution of a strongly basic titrant, such as sodium hydroxide, is used to titrate acids. Bases are titrated with standard solution of hydrochloric acid or some other strongly acidic titrant. Most commonly, the end point of an acid-base titration is detected by observing the colour change of an indicator. However, for more accurate results, electrochemical methods, such as pH-metry and conductometry are used to locate the equivalence point.

First we will discuss the basic theory of acids and bases and the principle of acid-base titrations. After that you will be introduced to the actual experiment in which you will use a pH- meter to determine the dissociation constant of (a weak acid) acetic acid.

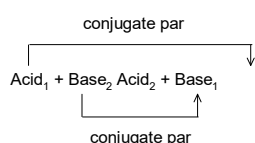
Expected Learning Outcomes

After reading this unit and performing the experiments you should be able to:

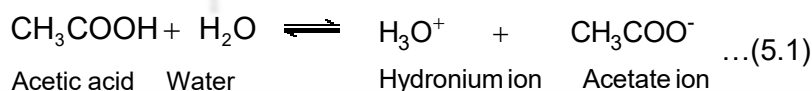
- ❖ derive equations useful in dissociation constant of an acid (K_a) and a base (K_b);
- ❖ follow the relationship between the equilibrium constant of weak acids and hydrogen ion concentration;
- ❖ understand the pH-meter;
- ❖ estimate acetic acid by using a pH-meter;
- ❖ determine the dissociation constant of the given weak acid by plotting pH titration curve; and
- ❖ take the precautions while doing an experiment on pH titration.

5.2 PRINCIPLE

Conjugate acids and bases are related by the gain or loss of one proton.
Conjugate pair



You have already studied about theories of acids and bases in Unit 7 of BCHCT 133. Please revisit the unit (section 7.4) to recall it. This will help you in understanding the basic principle of acid-base titrations. Discussions have been done on theory of Arrhenius (1884), which says an acid is any compound that releases protons, H^+ in water and a base is any compound that gives OH^- ions in water. Several limitations are there in this definition, such as, it applies only to aqueous solutions. Then, Bronsted and Lowry (1923) came with the next theory where an acid is a proton donor and a base is proton acceptor. Thus it could be applied to both aqueous and nonaqueous solutions. The third definition by Lewis (1938) stated that an acid is a substance that can accept an electron pair and a base is one that can donate an electron pair. Therefore the acid-base concept could be applied to many organic and inorganic reactions where there is no involvement of proton. Out of these definitions, we find Bronsted-Lowry definition the most suitable one for the purposes of explaining acid-base titrations. According to it an acid and a base react to give a conjugate base and a conjugate acid, respectively. The following reaction between acetic acid and water is an example for this. Here water acts as a base. The hydronium ions are the conjugate acid of water. The acetate ion which can donate an electron pair is the conjugate base of acetic acid.



These concepts will be useful to understand the following sections. Before proceeding further, answer the following SAQs.

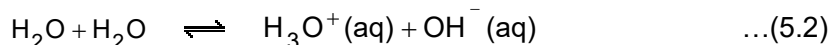
SAQ 1

Label the conjugate acid-base pair in the following reactions:

- (a) $HI + H_2O \rightleftharpoons H_3O^+ + I^-$
- (b) $CO_3^{2-} + H_2O \rightleftharpoons OH^- + HCO_3^-$
- (c) $CH_3COOH + H_2O \rightleftharpoons H_3O^+ + CH_3COO^-$
- (d) $NH_3 + HCl \rightleftharpoons NH_4^+ + Cl^-$

5.2.2 Ionisation of Water and the pH Concept

Water undergoes **autoionisation**, i.e., two molecules react giving hydronium and hydroxide ions; in this reaction one molecule acts as an acid, the other as a base, i.e.,



In Unit 7 of BCHCT 133, in Section 7.5, the ionic product of water has been derived and it is expressed as

$$K_w = [\text{H}_3\text{O}^+][\text{OH}^-] \quad \dots(5.3)$$

Since $[\text{H}_3\text{O}^+][\text{OH}^-]$ is the product of the ionic concentration, K_w is called the **ionic product** of water, or simply the **ionization constant** or **dissociation constant** of water (see Table 5.1 for its values).

Therefore in pure water and in all neutral aqueous solutions,

$$[\text{H}^+] = [\text{OH}]^- = \sqrt{1 \times 10^{-14}} = 1 \times 10^{-7} \text{ mol dm}^{-3}$$

The pH Concept

From the above you can see that it is quite cumbersome to express acidity or basicity in terms of hydrogen ion concentration or hydroxide ion concentration. These concentrations may range from relatively high values to very small ones, for example, 10 mol dm^{-3} to $10^{-11} \text{ mol dm}^{-3}$. In Unit 7 of BCHCT 133 you have already learnt about a very convenient concept called, pH, which was proposed by Sorensen (1909). He defined pH by the relationship,

$$\text{pH} = \log_{10} \frac{1}{[\text{H}^+]} = -\log_{10} [\text{H}^+] \quad \dots(5.4)$$

Similarly the hydroxide ion concentration is expressed as:

$$\text{pOH} = -\log[\text{OH}^-] \quad \dots(5.5)$$

Thus using this concept we can get from the equilibrium expression for the dissociation of water, $\log K_w = \log[\text{H}^+] + \log[\text{OH}^-]$

$$\text{p}K_w = \text{pH} + \text{pOH} \text{ (as } -\log K_w = \text{p}K_w)$$

Since $K_w = 1.0 \times 10^{-14}$, $\text{p}K_w = 14.00$. Thereby we get,

$$\text{pH} + \text{pOH} = 14.00 \quad \dots(5.6)$$

Do refer to section 7.5.2 of unit 7 of BCHCT 133 for calculation of pH. In Table 5.2, the summary of the values of pH, pOH in acidic, neutral and basic solution are given.

Table 5.2

	$[\text{H}^+]$	$[\text{OH}^-]$	pH	pOH
Acidic Solution	$>10^{-7}$	$<10^{-7}$	<7	>7
Neutral Solution	10^{-7}	10^{-7}	7	7
Basic Solution	$<10^{-7}$	$>10^{-7}$	>7	<7

Many other solvents like NH_3 also undergo autoionisation.

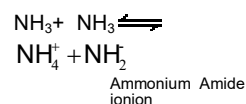


Table 5.1: Temperature dependence of K_w

Temperature (K)	$K_w (\text{mol}^2 \text{dm}^{-6})$
273	0.11×10^{-14}
298	1.00×10^{-14}
323	5.47×10^{-14}
373	51.3×10^{-14}

In an acid solution $(\text{H}^+) > (\text{OH}^-)$, i.e. $(\text{H}^+) > 10^{-7}$ and $(\text{OH}^-) < 10^{-7}$.
In a basic solution $(\text{OH}^-) > (\text{H}^+)$, i.e., $(\text{OH}^-) > 10^{-7}$ and $(\text{H}^+) < 10^{-7}$.

The notation pH was originally used for 'potential of hydrogen'. The 'p' notation can be applied to other quantities and always means "minus $\log_{10}$ of", for example:

$$\text{p}K_a = -\log_{10} K_a$$

$\text{p}K_b = -\log_{10} K_b$ and so on. pH gives a convenient scale for expressing $[\text{H}^+]$ of a solution.

The measure of the acidity of an aqueous solution is the actual concentration of hydrogen ions in the solution. The measure of basicity, on the other hand, is the actual concentration of hydroxide ions.

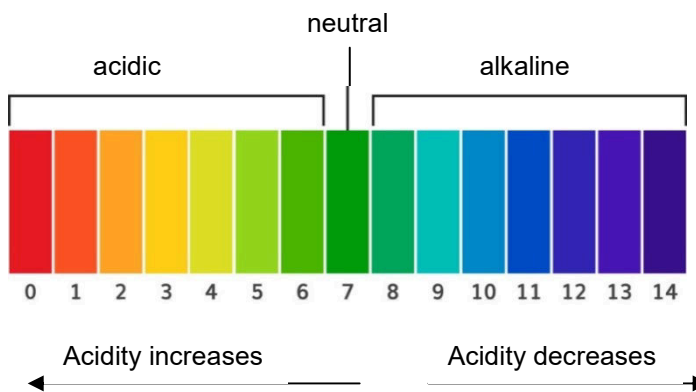


Fig. 5.1: pH scale.

From the above discussion you can see that acidity or basicity of a solution can be expressed conveniently in terms of pH. So far we have discussed the pH concept and ionic equilibrium of water. In the next section, we will discuss dissociation of weak acids and weak bases.

5.2.2 Dissociation of Weak Acids and Weak Bases

For a weak acid or a weak base, you must be knowing that these concentrations of hydrogen or hydroxide ions are always less in aqueous solution than the stoichiometric acid or base concentrations. The degree of dissociation or ionisation of the weak acid or base which is represented by the respective dissociation constant or ionisation constant gives to what extent it gives the respective ions.

Acetic acid, CH_3COOH , is a typical example of a weak acid:



The equilibrium expression for this reaction is

$$K = \frac{[\text{H}_3\text{O}^+][\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}][\text{H}_2\text{O}]} \quad \dots(5.7)$$

In dilute solution, the concentration of H_2O is almost the same as that of pure water.

Concentration of water may be taken as a constant for dilute solutions and included with K . That is,

$$K \times [\text{H}_2\text{O}] = K_a = \frac{[\text{H}_3\text{O}^+][\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]}$$

We observe from the values of K_a or K_b , that smaller the extent of ionisation the weaker is acid or the base. On the other hand, smaller the value of $\text{p}K_a$ or $\text{p}K_b$, the stronger is the acid or base.

where we have used K_a to represent the **acid dissociation constant** or **ionisation constant**.

In general, for any weak acid, HA , the simplified equation for the dissociation reaction can be written as, $\text{HA} \rightleftharpoons \text{H}^+ + \text{A}^-$

$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} \text{ mol dm}^{-3} \quad \dots(5.8)$$

Similarly for the base B , we have $\text{B} + \text{H}_2\text{O} \rightleftharpoons \text{BH}^+ + \text{OH}^-$

and
$$K = \frac{[\text{BH}^+][\text{OH}^-]}{[\text{H}_2\text{O}][\text{B}]}$$

Now, $K_b = K[\text{H}_2\text{O}]$

$$K_b = \frac{[\text{BH}^+][\text{OH}^-]}{[\text{B}]} \text{ mol dm}^{-3} \quad \dots(5.9)$$

where K_b is the base dissociation constant.

Similar to the hydrogen ion concentration and pH, K_a , K_b and $\text{p}K_a$, $\text{p}K_b$ values are also used to express relative strength of acids and bases. Examples of K_a , $\text{p}K_a$, K_b and $\text{p}K_b$ values for aqueous solutions are given in Table 5.3.

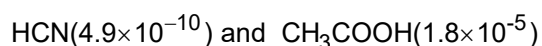
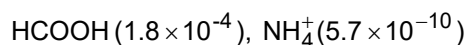
Table 5.3: K_a , $\text{p}K_a$, K_b and $\text{p}K_b$ values of some substances in aqueous solution (mainly at 298K).

	K_a	$\text{p}K_a$
Phenol	1.0×10^{-10}	10.0
<i>p</i> -Aminobenzoic acid	1.2×10^{-5}	4.92
Acetic acid	1.8×10^{-5}	4.74
Benzoic acid	6.46×10^{-4}	4.19
Lactic acid (2-Hydroxypropanoic acid)	1.4×10^{-4}	3.85
Nitrous acid	4.6×10^{-4}	3.34
2,2-Dichloroacetic acid	3.32×10^{-2}	1.48
	K_b	$\text{p}K_b$
Pyridine	1.48×10^{-9}	8.83
Ammonia solution (aqueous)	1.77×10^{-5}	4.75
Carbonate ion	2.1×10^{-4}	3.68
$(\text{CO}_3^{2-} + \text{H}_2\text{O} \rightleftharpoons \text{HCO}_3^- + \text{OH}^-)$		

Using Eq. 5.8 and 5.9, we can calculate equilibrium constant if pH of a solution of a given concentration of an acid or a base is known. If K_a or K_b is known, pH or pOH can be calculated for the given concentration of the acid or base. With this background we will discuss the theory of acid-base titrations in the next section.

SAQ 2

- (a) A sample of rain water from Delhi was recently found to have a pH of 4.
- Is this sample acidic or basic?
 - Determine the $[\text{H}^+]$ and $[\text{OH}^-]$ of the sample.
- (b) K_a values of some acids are given below. Rank these acids in terms of strength and also calculate their corresponding $\text{p}K_a$ values.



In titrations by the acid-base method, the titrant should be a strong acid or a strong base as an accurate end point cannot be detected by indicator method if both the titrand and the titrant are weak acid or weak bases, and *vice versa*.

5.2.3 Theories of Acid-Base Titrations

For strong acids and bases equivalence point does not depend on concentration. But for weak acids/bases the equivalence point depends on their concentration. If we titrate 0.1 mol dm^{-3} acetic acid (weak acid), with 0.1 mol dm^{-3} sodium hydroxide, a strong base, at equivalence point we will obtain 0.05 mol dm^{-3} sodium acetate. Eq.5.12 then yields the pH at the equivalence point $\text{pH} = 7.0 + 2.37 - 0.65 = 8.72$.

In acid-base titrations, as discussed earlier, a standard of an acid can be used for quantitative determination of bases (**acidimetry**), or a standard solution of a base can be used for quantitative determination of acids (**alkalimetry**). We generally use standard solution of a strong acid such as hydrochloric acid for acidimetry and that of a strong base such as sodium hydroxide for alkalimetry. But, these substances are not primary standards and, therefore, their standard solutions cannot be prepared from exact weights diluted to definite volumes; they must be standardized by titration. For standardizing HCl generally sodium carbonate and for sodium hydroxide we use oxalic acid or potassium hydrogenphthalate. The preparation of standard solutions and the method of standardization will be discussed in the experimental part.

You have studied earlier that for acid-base titrations, at a certain definite pH value, the equivalence point is reached, when the number of moles of OH^- or H^+ just react with all the H^+ or OH^- ions respectively.

The pH value at the equivalence point depends on the nature of the reacting substances, acid and base. For example, in case of a strong acid and a strong base, the reaction is completed at pH 7, therefore, equivalence point should be pH 7. However, in the case of titration of a weak acid and a strong base, due to the hydrolysis of the salt formed on the addition of the base, equivalence point is observed at $\text{pH} > 7$, and not at pH 7. For example, in the titration of acetic acid (0.1 mol dm^{-3}) with sodium hydroxide (0.1 mol dm^{-3}), the equivalence point is at $\text{pH} = 8.72$. In the case of the titration of a strong acid with a weak base, i.e., hydrochloric acid with sodium carbonate, the equivalence point will be at pH lower than 7. Thus, we can say, in different cases, titration will end at different pH values, depending on the nature and concentration of the reacting acid and base. The exact pH of the solution at the equivalence point in such titrations may be calculated from the ionisation constant of the weak acid (K_a) or the weak base (K_b) and the concentration (c) of the salt which is formed during the acid-base titration, using the following equations:

Weak acid and strong base

$$\text{pH} = \frac{1}{2}\text{p}K_w + \frac{1}{2}\text{p}K_a + \frac{1}{2}\log c \quad \dots(5.10)$$

Weak base and strong acid

$$\text{pH} = \frac{1}{2}\text{p}K_w - \frac{1}{2}\text{p}K_b - \frac{1}{2}\log c \quad \dots(5.11)$$

Weak acid and weak base

$$\text{pH} = \frac{1}{2}\text{p}K_w + \frac{1}{2}\text{p}K_a - \frac{1}{2}\text{p}K_b \quad \dots(5.12)$$

In sub-section 5.2.4 you will learn how titration curves can be used to find out the pH at the equivalence point.

Equivalence point may be detected with the help of acid-base indicators, pH-metry or potentiometry and conductometry. In the following section we will discuss the acid-base titration curves.

5.2.4 Acid-Base Titration Curves

If the pH of the titrand is monitored during a titration, a graph of pH against the amount of titrant added can be plotted. The curve so obtained is called the **acid-base titration curve** or **neutralisation curve**. Equivalence point can be detected with the help of these curves. Let us take the case of titration of a strong acid with a strong base first, e.g. 100 cm³ of 1 M hydrochloric acid when titrated with 1 M sodium hydroxide. As sodium hydroxide is added in small portions during titration, the pH will change. A pH-meter can help to measure the pH. The data of which is compiled in Table 5.4 and also presented graphically in Fig. 5.2.

Table 5.4 : Titration of 100 cm³ of 1M HCl with 1 M NaOH

NaOH added(cm ³)	pH	NaOH added(cm ³)	pH
0	0.0	100.1	10.7
50	0.5	100.2	11.0
75	0.8	100.5	11.4
90	1.3	101	11.7
98	2.0	102	12.0
99	2.3	110	12.7
99.5	2.6	125	13.0
99.8	3.0	150	13.3
99.9	3.3	200	13.5
100.0	7.0		

The slowly rising flattened region before the equivalence point is called the buffer region.

The significant feature of this curve is the very sharp and sudden change in pH near the equivalence point which in this case is at pH 7.

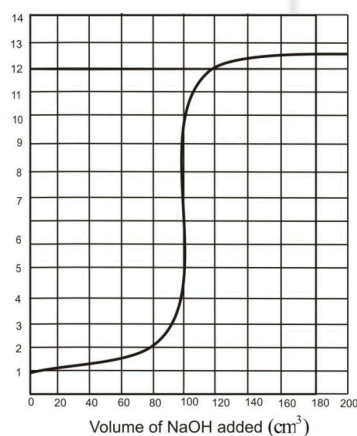


Fig. 5.2: Strong acid-strong base titration curve.

We now turn to titration of weak acids with strong bases. Suppose that 100 cm³ of 0.1M acetic acid solution is titrated with 0.1 M sodium hydroxide

solution. The results obtained, in this case, are summarized in Table 5.5 and are depicted graphically in Fig. 5.3.

A solution containing a substantial amount of both a weak acid and its conjugate weak base, resistant to pH change on a slight addition of an acid or a base, is a **buffer solution**.

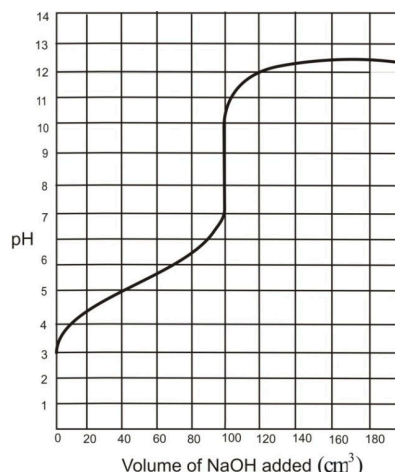


Fig. 5.3: Titration curve for a weak acid with a strong base.

The equivalence point in this case is at pH 8.7. As you know notice (Table 5.5) that during the addition of 25 cm³ to 90 cm³ of NaOH, the solution contains substantial amounts of both undissociated acetic acid (weak acid) and acetate ion (conjugate weak base). The solution, therefore, gets buffered in this region of the curve as shown in Fig. 5.3. The pH does not change very much as the volume of base added increases from 25 cm³ to 90 cm³. The influence of the strength of the acid or base, i.e., K_a or K_b on the pH change near the equivalence point is summarized in Figs. 5.4 and 5.5, respectively.

Table 5.5: Titration of a weak acid with strong base

NaOH added (cm ³)	pH	NaOH added (cm ³)	pH
0	2.9	99.9	7.7
10	3.8	100.0	8.7
25	4.3	100.2	10.0
50	4.7	100.5	10.4
90	5.7	101	11.7
99.0	6.7	125	12.0
99.5	7.0	150	12.3
99.8	7.7	200	12.5

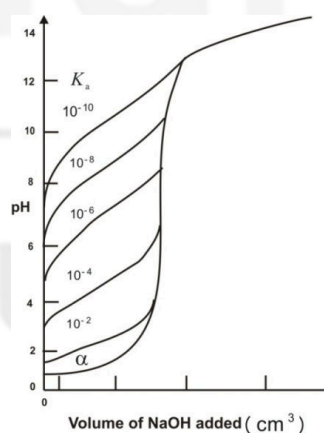


Fig. 5.4: The effect of K_a on the titration curves for weak acid with a strong base.

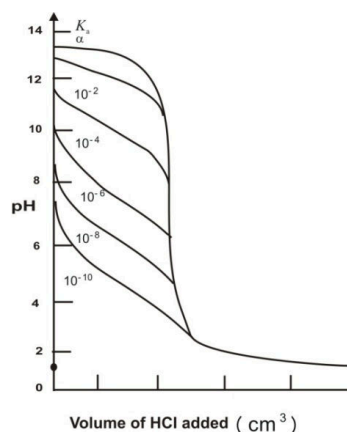
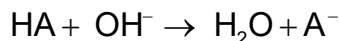


Fig. 5.5: The effect of K_b on the titration curves for weak bases with a strong acid.

So what can you conclude from Figs. 5.4 and 5.5? It is that different concentrations and strengths of acids or bases give rise to different shapes of titration curves.

Analyzing a Titration Curve-the importance of the pH at the half-equivalence point

We will take the case of the experiment which you have to perform in this unit, where a strong base (from a burette, for example) is added to a weak acid (in a beaker under the burette). The reaction which takes place is as:



From the shape of the curve we can derive the position of the equivalence point.

Consider three points during the titration:

1. Before any base (OH⁻) have been added:

If you take the moment which is just before addition of the strong base to the acid, then the flask would only have the acid HA.

2. When the equivalence point is reached:

At the equivalence point, the acid (HA) and the base (OH⁻) have been all used up and only the product A⁻ would be present in the beaker.

3. When half the base needed to get to the equivalence point have been added:

Half of the acid (HA) will remain, and half the acid will have been turned into A⁻. (its conjugate base) In other words, [HA] = [A⁻].

In this experiment you are going to perform a titration of a weak acid against a strong base and you will measure pH changes during titration by pH-meter. Thereby the pK_a value of acetic acid will be determined by plotting pH titration curve. When a weak acid, HA, is dissociated in water, the following equilibrium is setup.



Where, HA(aq) is the weak acid, H⁺(aq) is the hydrogen ion and A⁻ is the conjugated base. The equilibrium constant K_a for this reaction is called dissociation constant of the acid HA as in Eq. 5.8:

$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}$$

To calculate K_a we need to know [H⁺], [A⁻], and [HA]. The concentration of hydrogen ion, [H⁺], is determined directly by measuring the pH. The pH at the half neutralization of a weak acid can be determined by measuring the pH with a pH-meter after each addition of a strong base and creating a titration curve which resembles the one shown in the Fig. 5.6 below.

When equivalence point is reached, the pH can be read halfway to the equivalence point. Thereby the dissociation constant i.e. K_a of the acid can be calculated.

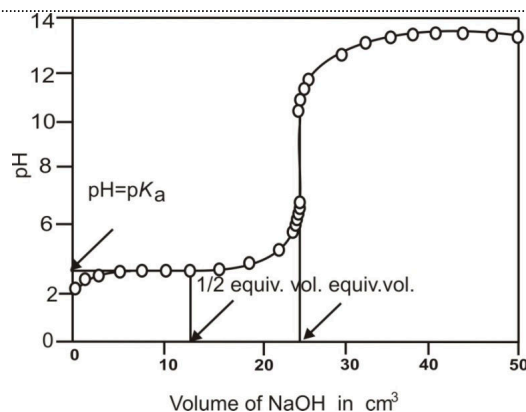


Fig. 5.6: pH titration curves: 0.10 Acetic acid with 0.10 NaOH.

In the next section we will discuss the working of a pH-meter.

SAQ 3

Indicate the buffer region in the titration curve given in Fig 5.2.

5.2.5 Working of a pH-Meter

A pH meter is used to measure the pH as base is added in small increments (called aliquots) to an acid solution.



Fig 5.7: pH-Meter.

In the previous course of BCHCT 135, in the unit on Electrochemistry you have learnt in details about the different types of electrodes. In the pH-meter you would need the glass electrode. The procedure of the working of pH-meter is given below:

Do not store the electrode in distilled water because this will cause ions to leak out of the glass bulb and thereby the electrode will become useless.

First of all switch on the instrument. Then wait for 10 minutes. Next take a well cleaned combined glass electrode. Connect it to the terminal marked 'input' at the back of the instrument. You have to dip the glass electrode into suitable buffer solution with known pH. The value of buffer solution displayed must be equal to the known pH. By this the pH-meter is calibrated. Next take the sample solution and note the pH value. When you have completed the experiment, leave the combined glass electrode in 0.1M HCl or tap water. (The details of glass electrode has already been discussed in Experiment 6 of BCHCL 134.)

5.3 REQUIREMENTS

Apparatus

pH-meter with combination electrode, volumetric flask (100 cm³), glass beaker(100 cm³), burette.

Chemicals

Oxalic acid solution, 0.10 M NaOH solution, 0.10 CH₃COOH solution, phenolphthalein indicator, standard buffers, distilled water.

During pH measurements, you have to completely dip the bulb of the glass / combination electrode into the solution.

5.4 PROCEDURE**Part A: Standardization of NaOH and Acetic acid**

1. Prepare oxalic acid solution (0.1 M) by dissolving appropriate amount of oxalic acid to 100 cm³ of distilled water in a volumetric flask of 100 cm³. Using this solution as primary standard, standardize NaOH solution (0.1N) using phenolphthalein indicator. Report your observations in observation Table I.
2. In the same way, use the above NaOH solution as secondary standard and standardize the acetic acid solution. Report your observations as in observation Table II.

Part B: Calibration of the pH-meter

Please request your counsellor to provide the instruction manual of the pH-meter used in the laboratory and study its operation. Please find below a general procedure for the calibration of pH-meter.

- i. After connecting the combination glass electrode to the pH-meter; switch on the pH-meter. Give about 10-15 minutes for it to stabilize.
- ii. Take the standard buffer solution of pH=7.0 in a clean beaker and place the combination glass electrode in this solution. Gently stir the solution and note the pH. If the value is not seven, you should adjust the calibration knob on the pH-meter to get the value of 7.0. Likewise the pH-meter should be pre-calibrated with standard buffers.

Part C: Determination of the K_a of Acetic Acid

1. NaOH is standardized by taking it in burette and titrating 10 cm³ acetic acid with phenolphthalein indicator to detect end point.
2. Next, take 25 cm³ acetic acid in a 100 cm³ beaker, to it add 20-25 cm³ of distilled water and dip the pH electrode to find the initial pH using pH-meter. The NaOH (which has been standardized) is taken in the burette and pH is recorded after addition of very small amounts (1-2 cm³). You have to stir well as soon as you add each aliquot of NaOH and towards end point only add amounts of 0.1-0.5 cm³. Remember that when you are almost at the end point, there will be a sudden change in the pH. The titration should be carried out till constant pH readings are observed in the range of 10-12. Record your observation as in observation Table III. Repeat the titration if you do not get a smooth titration curve for the pH readings.
3. Plot a graph of pH (y-axis) vs. volume NaOH added (x-axis). Determine the equivalence point from the graph. Find out the NaOH volume used in complete neutralization of acetic acid. From plotted curve find out the volume (in cm³) of NaOH and pH at half the neutralization of acetic acid. You can use the equation $pK_a = \text{pH}$ to calculate the value of K_a .

5.5 OBSERVATIONS

1. Preparation of Standard Oxalic Acid Solution in 100 cm³ Standard Flask

Molecular weight of oxalic acid = 126 g/mol

Mass of oxalic acid required to prepare (M/20) solution in 100 cm³ flask = 1.26 g

Mass of empty weight bottle = m_1 = ... g

Mass of bottle + oxalic acid = m_2 = ... g

Mass of bottle after transferring oxalic acid = m_3 = ... g

Mass of oxalic acid actually transferred = $w_2 - m_3 = w$ = ... g

Molarity of oxalic acid solution:

$$M_{\text{oxalic acid}} = \frac{m}{126} \times 10 = \dots\dots\dots M$$

2. Observation Table I: Standardization of NaOH.

S.No.	Volume of oxalic acid (cm ³)	Burette Reading		Volume of NaOH used (cm ³)
		Initial	Final	
1	10			
2	10			
3	10			

Volume of NaOH added at end point = V_{NaOH} = ...cm³

$$M_{\text{NaOH}} \times V_{\text{NaOH}} = M_{\text{oxalic acid}} \times V_{\text{oxalic acid}}$$

Molarity of NaOH = M_{NaOH} = ... M

Observation Table II: Standardization of Acetic Acid

S.No.	Volume of Acetic acid (cm ³)	Burette Reading		Volume of NaOH (cm ³)
		Initial	Final	
1	10			
2	10			
3	10			

Volume of NaOH added at the end point = V_{NaOH} = ...cm³

Molarity of Standardized NaOH = M_{NaOH}

$$M_{\text{NaOH}} \times V_{\text{NaOH}} = M_{\text{acetic acid}} \times V_{\text{acetic acid}}$$

Calculated molarity of Acetic acid = ... M

Observation Table III: Determination of the K_a of CH_3COOH

S.No.	Volume of NaOH added (cm^3)	pH
1	0.0	
2	2.0	
3	4.0	
4	6.0	
5	8.0	
6	10.0	
7	12.0	
8	14.0	
9	16.0	
10	18.0	
11	20.0	
12	22.0	
13	24.0	
14	24.5	
15	25.0	
16	26.5	
17	27.0	
18	27.5	
19	28.0	

Plot the graph of pH versus volume of NaOH which looks like Fig. 5.3.

5.6 CALCULATIONS

Volume of NaOH added to get equivalence point..... cm^3
(from plotted graph).

Volume of NaOH added to get half neutralization..... cm^3

pH at half neutralization

$$\text{p}K_a = \text{pH}$$

$$K_a = \text{antilog}(-\text{p}K_a) = \text{antilog}(-\text{pH})$$

$$K_a = \dots\dots\dots$$

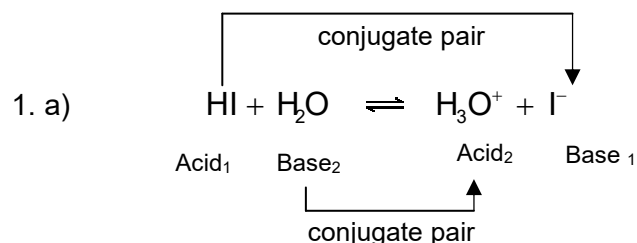
5.7 RESULTS

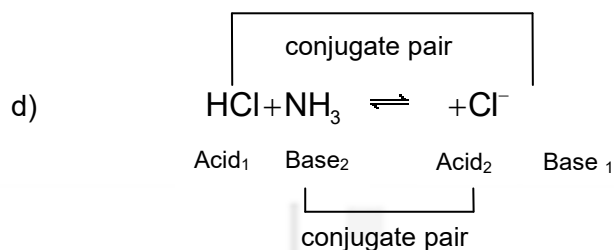
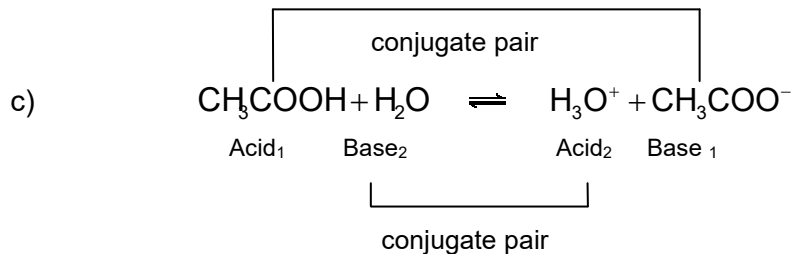
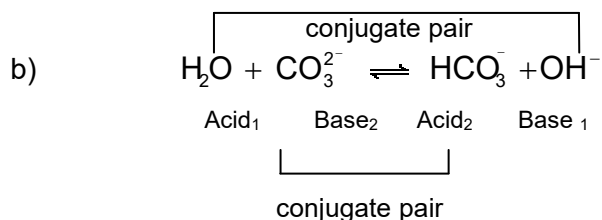
K_a value from half neutralization method =

K_a from Literature =

Percentage error =

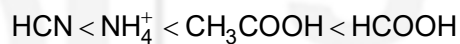
5.8 ANSWERS TO SAQs





2. a) (i) Acidic (ii) $(H^+) = 1 \times 10^{-4} \text{ mol dm}^{-3}$, $(OH^-) = 1 \times 10^{-10} \text{ mol dm}^{-3}$

b) Increasing order of acid strength:



$$pK_a \text{ of } HCOOH = -\log K_a$$

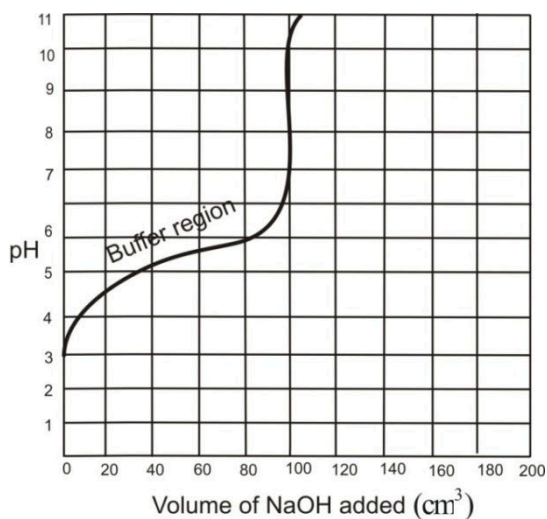
$$= -\log 1.8 \times 10^{-4}$$

$$= -(1.8 + 10^{-4})$$

$$= -(0.2553 - 4)$$

$$pK_a \text{ of } HCN = 9.31, pK_a \text{ of } NH_4^+ = 9.24, pK_a \text{ of } CH_3COOH = 4.74.$$

3. Buffer region in the titration curve of Fig. 5.2.



EXPERIMENT 6

CONDUCTOMETRIC TITRATIONS OF ACIDS AND BASES

Structure

6.1	Introduction	6.5	Observations
	Expected Learning Outcomes	6.6	Calculations
6.2	Principle	6.7	Result
6.3	Requirements		
6.4	Procedure		

6.1 INTRODUCTION

In the previous experiment you have learnt about the determination of dissociation constant of a weak acid by using a pH meter. In this experiment you are going to learn about and perform conductometric titrations between acids and bases. Conductometry i.e., the measurement of conductance of the titrating mixture is one of the common instrumental methods used for the determination of equivalence point of a titration. This method of determination of equivalence point becomes relevant when either the sample to be analysed is of low concentration or has a dark colour, as in industrial effluents. These do not permit the use of common visual indicators. You are going to learn about and perform two conductometric acid-base titrations here. The first one is between HCl (a strong acid) and NaOH (a strong base) while the second one is between CH_3COOH (a weak acid) and NaOH (a strong base). Though the principle is the same, the nature of titration curves is different in these cases. We would explain the reasons for the difference and you would be able to verify the same by actually performing the titrations.

In the next experiment you would learn about another instrumental method viz., potentiometry for the determination of equivalence point of acid base titrations.

Expected Learning Outcomes

After studying about and performing the experiment, you should be able to

- ❖ define conductometric titrations and state their importance;
- ❖ explain the nature of conductometric titration curve for a strong (or weak) acid and a strong base;
- ❖ prepare a standard solution of oxalic acid and use it to standardise a solution of sodium hydroxide;
- ❖ perform conductometric titration between HCl (or acetic acid) and NaOH;
- ❖ plot a graph of variation in conductance of solution in the course of the titration between HCl (or acetic acid) and sodium hydroxide;
- ❖ determine the equivalence point of the titration from the titration curve; and
- ❖ calculate the strength of given HCl (or acetic acid) solution.

6.2 PRINCIPLE

You know that the electrical conductance of a solution is a measure of current carrying capacity of the ions present in the solution. It is determined by the type and concentration of ions present in the solution. In the conductometric titrations we measure the conductance of solution being analysed as a function of the volume of titrant added and use the nature of its variation for the determination of equivalence point of the titration.

In a conductometric titration the ionic specie to be determined is made to react with a suitable reagent to convert it to a non-ionic form. For example, in the determination of acid solutions the base is the suitable reagent that converts the hydrogen ions of the acid solution into undissociated water molecules. In this process the ion being determined is replaced by another ion e.g., in the titration of HCl with NaOH, the hydrogen ions will be replaced by Na^+ ions. In order to get good results, it is advisable to use a reagent such that there is a significant difference in the conductivity of the ion being determined and the ion replacing it.

In a typical conductometric titration a measured volume of the solution to be determined (titrand) is taken in a beaker and a conductivity cell is dipped into it. The solution of titrant (reagent) is added from a burette into the titrand solution and the conductance readings corresponding to the various additions of the titrant are measured and recorded. These are then plotted against the volume of the titrant to obtain the titration curve.

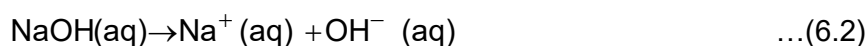
As you have learnt in Unit 5 of BCHCT-135 course that in the process of measurement of conductance, we measure the conductance of all the ions present in the column of solution contained within the electrodes of the conductivity cell. During the titration, the measured conductance changes mainly due to the change in the concentration of the ions in the region due to the reaction taking place. However, the dilution of the solution during the titration also affects the number of ions and hence the measured conductance. It is, therefore, advisable to use a titrant solution of a relatively higher concentration than that expected for the titrand. Generally, in conductometric titrations, the titrant (the solution in burette) is taken to be about ten times more concentrated than the titrand (solution in the conical flask / beaker) so as to minimise the dilution effect.

HCl versus NaOH titration

In the titration of HCl (a strong acid) versus NaOH (a strong base) initially the titrand solution will consist of hydrogen ions and chloride ions obtained as a result of ionisation of the acid. The conductance of the solution will be high due to the presence of hydrogen ions having extremely high (due to small size and Grotthuss mechanism) conductivity.



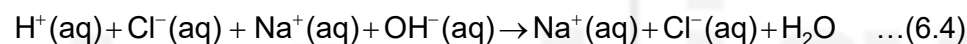
When we add a solution of sodium hydroxide to the solution of hydrochloric acid, it would provide sodium ions and hydroxide ions obtained from its dissociation as given below.



Of these two ions, the hydroxide ions have high conductance whereas sodium ions are less conducting. The hydroxide ions would neutralise the hydrogen ions and produce water molecules that are practically unionised.



The overall reaction can be given as



This means that the addition of NaOH solution to HCl solution removes some hydrogen ions from the solution and replace them with sodium ions. As the sodium ions have much less conductance as compared to hydrogen ions, the conductance of the solution decreases. Adding more and more amount of NaOH solution would neutralise more and more of hydrogen ions and the conductance of the solution would continue to decrease. This trend would continue till all the hydrogen ions are neutralised i.e., the equivalence point is reached.

Adding a drop of NaOH solution at this stage would provide additional sodium ions and hydroxide ions. As a consequence, the conductance of the solution would start to increase. This trend would continue with addition of more and more of NaOH solution. A graph between the conductance of the solution and the volume of NaOH solution, will give the titration curve; Fig. 6.1.

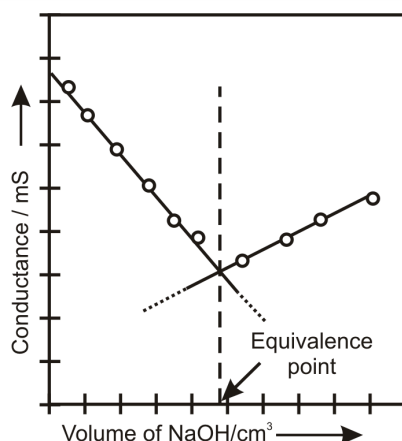
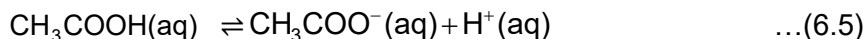


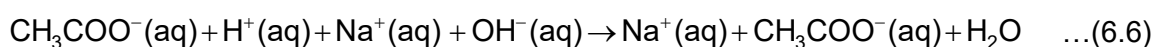
Fig. 6.1: Schematic representation of titration curve for HCl versus NaOH conductometric titration.

Acetic acid versus NaOH titration

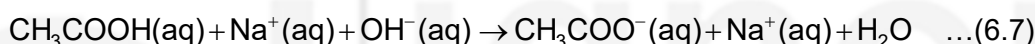
In the conductometric titration between acetic acid (a weak acid) and NaOH (a strong base) initially the titrand solution will consist of small amount hydrogen ions and acetate ions obtained as a result of ionisation of the acetic acid as given below.



You know that acetic acid is a weak acid and ionises only slightly in solution. As a consequence, the conductance of the solution will be low. On adding a few drops of sodium hydroxide in the beginning of the titration the hydrogen ions present in the solution will combine with the hydroxide ions and give undissociated water molecules.



In this process, the fast-moving hydrogen ions are replaced by slower sodium ions and the conductance decreases a bit. Once all the free hydrogen ions are neutralised, the added sodium hydroxide reacts with the unionised acetic acid as follows to form the salt, sodium acetate.



As a result, the number of ions in the solution and hence the conductance of the solution would increase. This process will continue with the addition of more and more of sodium hydroxide and the conductance would continue to increase till the equivalence point is reached. Once all the acetic acid is neutralised (at equivalence point) any further addition of sodium hydroxide will provide additional sodium and hydroxide ions and the conductance of the solution will increase further. As the hydroxide ions have quite a high conductance, the increase in the conductance will be much faster now. This change in the rate of increase of the conductance of the solution indicates the equivalence point of the titration. A typical conductometric titration curve for the titration of acetic acid and sodium hydroxide is given in Fig. 6.2.

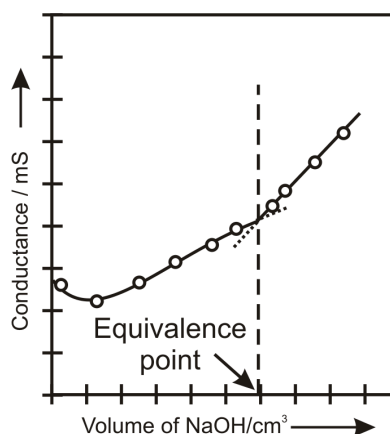


Fig. 6.2: Schematic conductometric titration curve for acetic acid versus NaOH titration.

6.3 REQUIREMENTS

Apparatus		Chemicals
Conductometer	1	Oxalic acid
Conductivity cell	1	Acetic acid
Volumetric flask (100 cm ³)	1	Hydrochloric acid
Burette (50 cm ³)	1	Sodium hydroxide
Pipette (10 cm ³ and 20 cm ³)	1 each	Phenolphthalein
Conical flask (100 cm ³)	1	Conductivity water
Burette stand with clamp	1	
Beaker 50 cm ³	1	
Weighing bottle	1	
Analytical balance	1	
Weight box	1	
Funnel	1	

Solutions provided:

The following would be provided by your counsellor.

1. **Conductivity water:** It is repeated distilled water having electrical conductivity less than 0.01 mS m⁻¹. It is desirable that it is boiled shortly before use to minimise the CO₂ content.
2. **Sodium hydroxide solution (~0.1 M):** It is prepared by dissolving ~ 4 g of sodium hydroxide in about 150-200 cm³ of conductivity water and diluting it to 1 litre.
3. **Hydrochloric acid (~0.01 M):** It is prepared by adding about 1.0 cm³ of concentrated hydrochloric acid to about 100 cm³ of conductivity water and diluting it to 1 litre.
4. **Acetic acid solution (~0.01M):** It is prepared by adding about 0.6 cm³ of glacial acetic to 100 cm³ of conductivity water and diluting it to 1 litre.
5. **Phenolphthalein indicator:** It is prepared by dissolving 1 g of the reagent in 100 cm³ of ethanol and adding 100 cm³ of water to it. If a precipitate is formed, it is filtered.

6.4 PROCEDURE

You are going to perform two conductometric titrations here. The procedure for both of these is same as given below. You will first perform the conductometric titration for hydrochloric acid with NaOH. The procedure consists of the following steps.

- a) Preparation of standard solution of oxalic acid

- b) Standardisation of sodium hydroxide solution by titrating against standard oxalic acid solution
- c) Calibrating the conductometer
- d) Performing conductometric titration of the given solution of hydrochloric acid (or acetic acid) with standardised solution of sodium hydroxide
- e) Calculation of the concentration of given hydrochloric acid (or acetic acid) solution

A. Experiment 6.1: Conductometric titration of HCl with NaOH

Follow the procedure given below in a stepwise manner to determine the concentration of given solution of hydrochloric acid solution conductometrically.

a) Preparation of 100 cm³ of standard solution of 0.05 M oxalic acid

- i) As you know, to prepare a standard solution we need to first calculate the amount of the substance required. The amount of oxalic acid ($M_m = 126 \text{ g mol}^{-1}$) required to prepare 100 cm³ of 0.05 M solution of oxalic acid would be:

$$\text{mass (in g)} = \frac{MV M_m}{1000} = \frac{0.05 \text{ mol dm}^{-3} \times 100 \text{ cm}^3 \times 126 \text{ g mol}^{-1}}{1000 \text{ cm}^3 \text{ dm}^{-3}} = 0.63 \text{ g}$$

- ii) Therefore, weigh about 0.65 g of oxalic acid on a rough weighing balance and transfer it to a clean dry weighing bottle and accurately weigh the weighing bottle with oxalic acid.
- iii) Transfer the weighed oxalic acid to a clean volumetric flask of 100 cm³ capacity through a funnel.
- iv) Weigh the weighing bottle (may contain some oxalic acid) again and find the exact mass of oxalic acid transferred by subtracting this mass from the mass of the weighing bottle plus oxalic acid (step ii).
- v) Dissolve oxalic acid by adding about 30–40 cm³ of distilled water in the volumetric flask. Once dissolved, make the volume up to the mark with distilled water.

b) Standardisation of sodium hydroxide solution by titrating against standard solution of oxalic acid

- i) Fill the burette with given solution of sodium hydroxide with the help of a funnel and mount the burette on the burette stand. Note the reading on the burette and record it in the Observation Table 6.1 under the initial reading column.
- ii) Carefully pipette out 10 cm³ of the standard solution of oxalic acid and transfer it to a clean 100 cm³ conical flask. Add one to two drops of phenolphthalein indicator to it.
- iii) Titrate the coloured solution with NaOH using constant swirling till a persistent pink colour is obtained. The colour should not fade on shaking the solution. Record the reading in the Observation Table 6.1, under the final reading column.

- iv) Repeat the titration to get at least two concordant readings and record the same in the Observation Table 6.1.

c) Calibrating the conductometer

- i) Connect the conductometer to the power mains and allow it to stabilise for about 10-15 minutes.
- ii) Follow the instructions from the operating manual accompanying the instrument and calibrate the instrument. (*Your counsellor will help you in this step*)

d) Performing, conductometric titration of solution of hydrochloric acid with standardised solution of sodium hydroxide

- i) Before starting the titration, you should determine the drop volume of your burette. For this, adjust the burette (containing NaOH solution) at a certain value (say 0) and note it. Then carefully drain out 100 drops of the solution into a beaker and note the burette reading again and record it. Divide the difference of the two readings by 100 to get the drop volume.
- ii) Pipette out 20 cm^3 of the solution of hydrochloric acid in a 50 cm^3 beaker and dip the conductivity cell connected to the conductometer into it. (Ensure that the electrodes of the conductivity cell are completely dipped in the solution. If not, then pipette out 10 cm^3 more of HCl solution and add to the beaker.)
- iii) Read the conductance of the solution from the conductometer (Please use the instructions provided in the operation manual for reading the conductance) and record them in the Observation Table 6.2.
- iv) Add 2 drops of NaOH solution from the burette and stir the solution well with the help of a glass rod. Read the conductance, keeping the stirrer above the solution and record the value in Observation Table 6.2.
- v) Keep repeating the step iv) and record your observations in the Observation Table-6.2. You will observe that the conductance value keeps on decreasing with every addition of NaOH solution. After a certain stage, the conductance of solution will start increasing (due to excess of NaOH). Take 4-5 readings more after the conductance reading starts increasing.
- vi) Plot a graph for the conductance of the solution versus the volume (in number of drops) of NaOH on a graph paper.
- vii) Determine the equivalence point from the graph. This is obtained by noting down the point at which the curve, for the decreasing conductance due to the neutralisation of hydrochloric acid and the one for increasing conductance due to the excess of NaOH, meet. For this extrapolate the two lines and note the point at which the two intersect.

e) Calculation of the concentration of hydrochloric acid

Convert the volume (in number of drops) of NaOH solution at the equivalence point to its volume in cm^3 by multiplying with drop volume. Calculate the concentration of the given solution of hydrochloric acid by substituting the

concentration of sodium hydroxide along with the volumes of the sodium hydroxide and hydrochloric acid used in the molarity equation. Report the result.

Experiment 6.2: Conductometric titration of CH_3COOH with NaOH

The steps **a to c** of the procedure are the same as given above for the titration of HCl . You do not need to repeat them. Follow the procedure given below in a step wise manner to determine the concentration of given solution of acetic acid.

d) Performing conductometric titration of the given solution of acetic acid with standardised solution of sodium hydroxide.

- i) Pipette out 20 cm^3 of the solution of acetic acid in a 50 cm^3 beaker and dip the conductivity cell connected to the conductometer into it. (Ensure that the electrodes of the conductivity cell are completely dipped in the solution. If not, then pipette out 10 cm^3 more of HCl solution and add to the beaker.
- ii) Read the conductance of the solution from the conductometer (Please use the instructions in the operation manual provided with the instrument for reading the conductance) and record it in the Observation Table 6.3.
- iii) Add 1 drop of solution of NaOH from the burette, stir the solution well with the help of a glass rod, read the conductance and record its value in the Observation Table 6.3.
- iv) Keep repeating the step iii) by using 2 drops of NaOH every time and record your observations in the Observation table 6.3. You will observe that the conductance value gradually keeps on increasing with every addition of NaOH . After a certain stage it will start increasing sharply (due to excess of NaOH). Take 4-5 readings more after the conductance reading starts increasing sharply.
- v) Plot a graph for the conductance versus volume (in number of drops) of NaOH on a graph paper.
- vi) Determine the equivalence point from the graph by noting down the point at which the curve for the neutralisation of acetic acid and for the excess of NaOH meet. For this, extrapolate the two lines and note the point at which the two intersect.

e) Calculation of the concentration of acetic acid

Convert the volume (in number of drops) of NaOH solution at the equivalence point to its volume in cm^3 by multiplying with drop volume of the burette. Calculate the concentration of the given solution of hydrochloric acid by substituting, the concentration of sodium hydroxide along with the volumes of the sodium hydroxide and acetic acid used, in the molarity equation. Report the result.

6.5 OBSERVATIONS

a) Preparation of 100 cm^3 of standard solution of 0.05 M oxalic acid

Mass of weighing bottle + oxalic acid = $m_1 \text{ g}$ = ...g

Mass of weighing bottle (after transferring oxalic acid) = $m_2 \text{ g}$ = ... g

b) Standardisation of the given solution of sodium hydroxide by titrating against standard solution of oxalic acid

Volume of standard solution of oxalic acid taken, $V_O = \dots \text{cm}^3$

Solution in the burette: Sodium hydroxide

Indicator used: Phenolphthalein

Observation Table 6.1: Standardisation of sodium hydroxide

S.No.	Volume of oxalic acid (in cm^3)	Burette reading		Titer value (in cm^3) (Final-initial reading)
		Initial	Final	
1				
2				
3				
Concordant reading				

D. Conductometric titration of hydrochloric acid (or acetic acid) with sodium hydroxide solution

Record the following readings for the calculation of drop volume of burette

Initial reading of the burette: $R_1 = \dots$

Burette reading after draining 100 drops of solution, $R_2 = \dots$

$$\text{Drop volume} = \frac{(R_2 - R_1)}{100} = \dots \text{cm}^3$$

Volume of hydrochloric acid solution taken in beaker $V_H = \dots \text{cm}^3$

Volume of acetic acid solution taken in beaker $V_A = \dots \text{cm}^3$

Solution in the burette: Sodium hydroxide

Molarity of sodium hydroxide = $\dots M$ (To be obtained by standardisation)

Observation Table 6.2: Conductometric titration of HCl with NaOH

S.No.	Number of drops of NaOH	Conductance (in mS cm^{-1})	S.No.	Number of drops of NaOH added	Conductance (in mS cm^{-1})
1			16		
2			17		
3			18		
4			19		
5			20		
6			21		
7			22		
8			23		
9			24		

10			25		
11			26		
12			27		
13			28		
14			29		
15			30		

Observation Table 6.3: Conductometric titration of acetic acid with NaOH

S.No.	Number of drops of NaOH	Conductance (in mS cm ⁻¹)	S.No.	Number of drops of NaOH added	Conductance (in mS cm ⁻¹)
1			16		
2			17		
3			18		
4			19		
5			20		
6			21		
7			22		
8			23		
9			24		
10			25		
11			26		
12			27		
13			28		
14			29		
15			30		

6.6 CALCULATIONS

Perform the following calculations based on the observations recorded above. You need to perform the calculations stepwise. Let us begin with step a).

a) Preparation of 100 cm³ of standard solution of 0.05 M oxalic acid

Molarity of the prepared standard solution of oxalic acid can be calculated as follows:

Mass of weighing bottle + oxalic acid = m_1 g = ...g

Mass of weighing bottle (after transferring oxalic acid) = m_2 g = ... g

Amount of oxalic acid transferred = $m_1 - m_2 = m$ g = ... g

Molar mass (M_m) of oxalic acid = 126 g mol⁻¹

Volume of solution of oxalic acid prepared = 100 cm³

$$\text{Molarity of oxalic acid solution} = M_o = \frac{m \times 1000}{126 \times 100} = \frac{10 m}{126} = \dots\dots\dots M$$

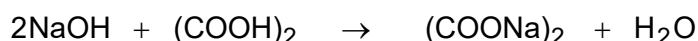
b) Standardisation of the given solution of sodium hydroxide by titrating against standard solution of oxalic acid.

Volume of standard solution of oxalic acid taken in conical flask, $V_o = \dots \text{cm}^3$

Volume of solution of NaOH used (concordant value from Observation Table 6.1) $= V_N = \dots\dots \text{cm}^3$

The concentration of the given solution of sodium hydroxide can be determined as follows:

The reaction involved in the titration:



Molarity equation: $M_N V_N = 2M_o V_o$

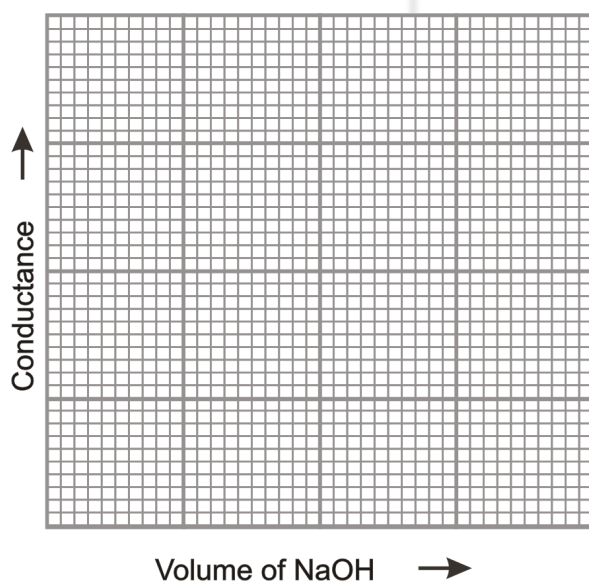
The molarity of solution of sodium hydroxide = $M_N = \frac{2M_o V_o}{V_N} = \dots\dots\dots M$

$$= M_N = \dots\dots\dots M$$

Determination of equivalence point

HCl versus NaOH titration

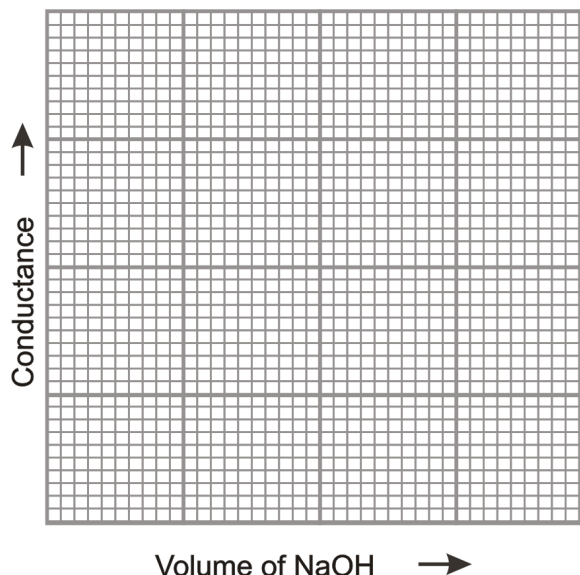
Plot a graph between the measured conductance (on y-axis) and the volume of NaOH (in number of drops; on x-axis) using the data from Observation Table 6.2. The equivalence point is obtained by extrapolating the lines obtained for decreasing conductance due to neutralisation of HCl and the one for increasing conductance due to excess of NaOH. The intersection of these lines gives the equivalence point. Note the volume of solution of NaOH at the equivalence point by dropping a perpendicular from the equivalence point on to the x-axis.



Graph-I: Conductometric titration of HCl versus NaOH.

CH₃COOH versus NaOH titration

Plot a graph between the measured conductance (on y-axis) and the volume of NaOH (in number of drops; on x-axis) using the data from Observation Table 6.3. Determine the equivalence point by extrapolating the lines obtained for gradual increase in conductance due to neutralisation of acetic acid and the one for increasing conductance due to the excess of NaOH. The intersection of these lines gives the equivalence point. Note the volume of NaOH at the equivalence point by dropping a perpendicular from the equivalence point on to the x-axis.



Graph-II: Conductometric titration of CH₃COOH versus NaOH.

Calculation of the concentration of hydrochloric acid

Volume of solution of hydrochloric acid taken in beaker $V_H = \dots \text{cm}^3$

Molarity of solution of sodium hydroxide (obtained from Standardisation),
 $M_N = \dots M$

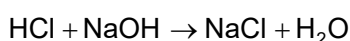
Volume of solution of sodium hydroxide used (in number of drops) [from graph-I] = ...

Volume of solution of sodium hydroxide used (in cm^3) =

$$(\text{Number of drops} \times \text{drop volume}) = V_N = \dots \text{cm}^3$$

Let molarity of the given solution of hydrochloric acid = M_H

The reaction involved in the titration:



Molarity equation: $M_H V_H = M_N V_N$

The molarity of hydrochloric acid = $M_H = \frac{M_N V_N}{V_H}$

Substituting the values, the molarity of hydrochloric acid = $M_H = \dots M$

Strength of the given solution of hydrochloric acid = molarity $\times$ molar mass

$$= M_H \text{ mol dm}^{-3} \times 36.5 \text{ g mol}^{-1} = \dots\dots\text{g dm}^{-3}$$

Calculation of the concentration of acetic acid

Volume of solution of acetic acid taken in beaker $V_A = \dots\text{cm}^3$

Molarity of solution of sodium hydroxide (obtained from standardisation),

$$M_N = \dots M$$

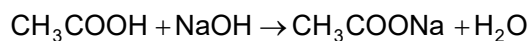
Volume of solution of sodium hydroxide used (in number of drops) [from graph-II]
=.....

Volume of solution of sodium hydroxide used (in cm^3)=

$$(\text{Number of drops} \times \text{drop volume}) = V_N = \dots\text{cm}^3$$

Let molarity of acetic acid = M_A

The reaction involved in the titration:



Molarity equation: $M_A V_A = M_N V_N$

The molarity of acetic acid = $M_A = \frac{M_N V_N}{V_A}$

Substituting the values, the molarity of acetic acid = $M_A = \dots\dots M$

Strength of the given solution of acetic acid = molarity $\times$ molar mass

$$= M_A \text{ mol dm}^{-3} \times 60 \text{ g mol}^{-1} = \dots\dots\dots\text{g dm}^{-3}$$

6.7 RESULTS

1. The strength of given solution of hydrochloric acid determined by conductometric titration with NaOH is found to be = $\dots\dots\text{g dm}^{-3}$.
2. The strength of given solution of acetic acid determined by conductometric titration with NaOH is found to be = $\dots\dots\text{g dm}^{-3}$.

EXPERIMENT 7

POTENTIOMETRIC TITRATIONS OF ACIDS AND BASES

Structure

7.1	Introduction	7.4	Procedure
	Expected Learning Outcomes	7.5	Observations
7.2	Principle	7.6	Calculations
7.3	Requirements	7.7	Result

7.1 INTRODUCTION

In the previous experiment you have learnt about conductometric titrations of acids and bases. In these, the variation of conductance of the solution with the progress of titration was followed and used to determine the equivalence point. In the present experiment, you would learn about another instrumental method used for the determination of equivalence point in acid-base titrations. This method is called **potentiometry**. In this method, we follow the potential of the galvanic cell constructed from the solution being analysed, with the progress of the titration to get the equivalence point. Like conductometry, potentiometry is also important in the analysis of solutions that are deeply coloured or turbid for which suitable visual indicators are not available. You are going to learn about and perform two potentiometric acid-base titrations. The first one is between HCl (a strong acid) and NaOH (a strong base) while the second one is between CH_3COOH (a weak acid) and NaOH (a strong base).

In the next experiment you would learn about systematic qualitative analysis of organic compounds possessing different functional groups

Expected Learning Outcomes

After studying about and performing this experiment, you should be able to

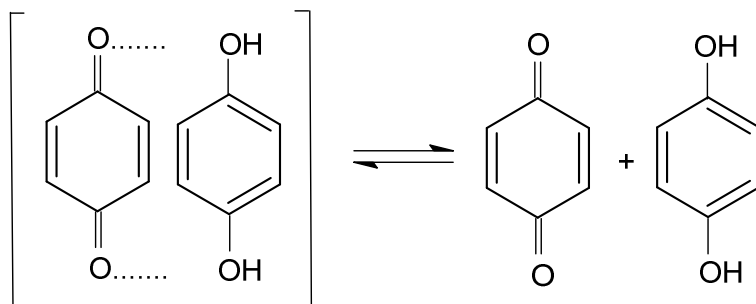
- ❖ define potentiometric titrations and state their importance;
- ❖ explain the nature of potentiometric titration curve for a strong (or weak) acid and a strong base;

- ❖ prepare a standard solution of oxalic acid and use it to standardise a solution of sodium hydroxide;
- ❖ perform potentiometric titration between HCl (or acetic acid) and NaOH;
- ❖ plot a graph of variation in cell emf in the course of the titration between HCl (or acetic acid) and sodium hydroxide;
- ❖ determine the equivalence point of the titrations from the titration curve by plotting derivative plots; and
- ❖ calculate the strength of given HCl (or acetic acid) solution.

7.2 PRINCIPLE

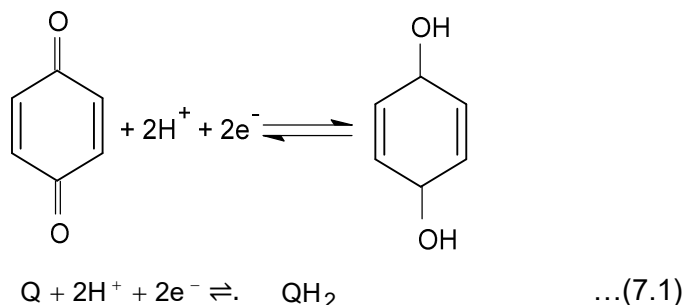
As mentioned above, sometimes it may not be possible to perform a titration using a visual indicator due to the sample solution being deeply coloured or turbid that may mask the colour change of the visual indicator. In such cases, an alternative process of titration is followed with the help of an instrument that can monitor the change in some physical property of the solution. The potential of an electrode set up using the test solution is one such physical property and potentiometer is the instrument. These titrations are called **potentiometric titrations**. You know that in a typical acid-base titration the concentration of hydrogen ions changes with the progress of the titration. Therefore, any method that can effectively measure the concentration of the hydrogen ions in solution can be used to follow the progress of an acid-base titration. You have learnt in your earlier classes as well as in Unit 7 and 8 of BCHCT-135 course that if we dip an electrode reversible to hydrogen ions, in an aqueous solution, the electrode potential is proportional to the concentration of hydrogen ions. Three such electrodes are commonly used for the determination of hydrogen ion concentration (hence pH) of the solution. These are hydrogen electrode, glass electrode and quinhydrone electrode.

Of these, the hydrogen electrode though effective, is difficult to set up and is avoided for routine measurements. Quinhydrone electrode is relatively inexpensive and easy to set up. In this method, we add the compound called quinhydrone (represented as Q.QH₂) to the solution to be analysed.



Quinhydrone is sparingly soluble in water and produces equal concentrations of the oxidised species, quinone (Q), and the reduced species, hydroquinone (QH₂). An inert platinum electrode dipped in a solution containing a mixture of

quinone and hydroquinone generates a redox electrode called the **quinhydrone electrode** that is reversible to hydrogen ions. The electrode reaction involves transfer of electrons between the quinone and hydroquinone and can be represented as:



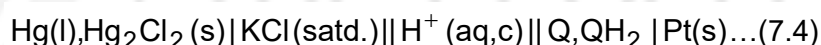
The potential of the quinhydrone electrode is given by the following expression

$$E_{Q|QH_2} = E_{Q|QH_2}^0 - \frac{RT}{2F} \ln \frac{[QH_2]}{[Q][H^+]^2} \quad \dots(7.2)$$

At 298 K, the standard potential of the quinhydrone electrode is found to be 0.6994 V. Further, the amounts of the oxidised and reduced forms are equal. Using these facts, the equation (7.2) simplifies to

$$E_{Q|QH_2} = (0.6994 - 0.0592 \text{ pH})V \quad \dots(7.3)$$

Thus, we see that the potential of the quinhydrone electrode depends on the pH of the solution i.e., it decreases with an increase in the pH of the solution. Now, since we cannot measure the potential of a single electrode we combine it with a standard electrode (typically saturated calomel electrode (SCE) or Ag-AgCl electrode) and construct a galvanic cell. The cell so obtained can be represented as



The cell emf, E_{cell} will be given as

$$E_{\text{cell}} = E_R - E_L \quad \dots(7.5)$$

The electrode potential of saturated calomel electrode at 298 K is 0.2682 V. Substituting the values in Eq. (7.3), we get

$$E_{\text{cell}} = (0.6994 - 0.0592 \text{ pH})V - 0.2682V = (0.4312 - 0.0592 \text{ pH})V \dots(7.6)$$

Thus, you can see that the emf of the galvanic cell obtained by combining quinhydrone electrode formed from the test solution and the saturated calomel electrode is related to the pH of the test solution. It decreases with an increase in the pH of the solution. The equation can equally well be represented in terms of the concentration of hydrogen ions as follows

$$E_{\text{cell}} = (0.4312 + 0.0592 \log[H^+])V \quad \dots(7.7)$$

In a typical potentiometric acid-base titration, we take the solution of the acid in a beaker; add a pinch of quinhydrone to it and insert a platinum wire into it to construct quinhydrone electrode. This is then coupled through a salt bridge with a reference electrode like laboratory saturated calomel electrode (SCE) or

Ag-AgCl electrode to form a galvanic cell. The emf of the cell so obtained is then measured, as a function of the volume of the titrant, using a potentiometer. The data of cell emf as a function of volume of titrant is then plotted to get the potentiometric titration curve.

Thus, a typical experimental set up of the galvanic cell for potentiometric titration consists of three components. These are: an indicator electrode, a reference electrode, and the salt bridge. The indicator electrode is that whose potential varies with variations in the concentration of the sample being analysed; in our case the quinhydrone electrode is the indicator electrode. The reference electrode, on the other hand is an electrode having a known potential. Its potential remains constant at constant temperature and does not depend on the composition of the solution being analysed; SCE or Ag-AgCl in our case are the reference electrodes. It is important to note that while setting up cell for potentiometric titration, the reference electrode is always taken as the left-hand electrode. The purpose of the third component, viz., the salt bridge is to prevent the mixing up of components of test solution and the reference electrode. The experimental set up for the potentiometric measurement of cell emf is given below in Fig. 7.1.

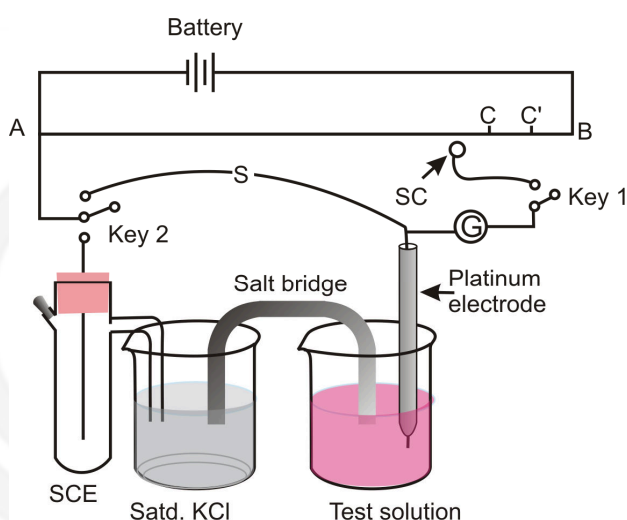


Fig. 7.1: Schematic representation of experimental set up for measurement of emf of a cell.

The emf of the cell is measured by a potentiometer which consists of a uniform resistance wire (AB), a battery with emf greater than that of the cell being measured, a standard cell (S) of known emf, two keys, a galvanometer, and a sliding contact (SC). These are connected as shown in Fig. 7.1.

To measure the cell emf, the cell for which the emf is to be measured is brought into the circuit by suitably using key 2 (it is a two way key) and connecting the other end of the cell to a sliding contact (SC) through a galvanometer G. The position of the sliding contact is varied to get the null point (when there is no deflection in the galvanometer) say at point (C). The point C is noted; the cell emf in this case is proportional to the length AC. Then the standard cell of known emf is brought into the circuit by changing the position of key 2. The same process is repeated to get a new null point, say at point C'. The emf of standard cell will be proportional to the length AC'. We can then write,

$$\frac{E_{\text{cell}}}{E_s} = \frac{AC}{AC'} \Rightarrow E_{\text{cell}} = E_s \times \frac{AC}{AC'}$$

However, now a days, most of the laboratories use direct reading potentiometer. Typically, commercially available digital pH meters are used as potentiometers without loosing much of the accuracy. These are quite convenient to set up and use. Also, if we use a dip type saturated calomel electrode then the set up becomes much simpler. We do not need to use the salt bridge as well as second beaker containing KCl. We can straightway dip the dip type SCE into the test solution itself. The experimental set up for potentiometric titration then simplifies to the one given in Fig. 7.2.

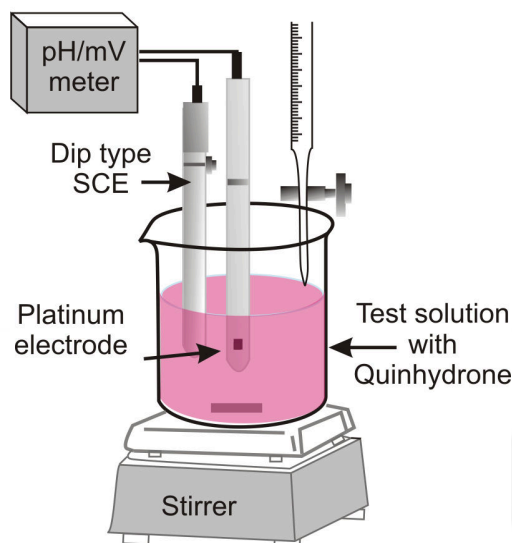


Fig. 7.2: Schematic diagram showing experimental set up for potentiometric titration using pH/mV meter and dip type saturated calomel electrode.

HCl versus NaOH titration

As per Eq. (7.7), the cell emf and the concentration of hydrogen ions are related as

$$E_{\text{cell}} = (0.4312 + 0.0592 \log [H^+]) V$$

In the titration of HCl versus NaOH, to begin with the concentration of hydrogen ions will be large and the E_{cell} will have a certain value. With the progress of the titration of HCl with NaOH, the concentration of hydrogen ions would keep on decreasing, therefore the cell emf would gradually decrease. Near the equivalence point the concentration of hydrogen ions decreases drastically leading to a sharp decrease in emf. After the equivalence point, once again the change in the emf value becomes gradual. These changes can be represented in terms of a titration curve- a plot of variation in cell emf with the volume of the titrant.

The nature of titration curve will depend on whether the acid is strong or weak; it also depends on the nature of base involved. For weak acids (or bases) the nature of titration curve would also depend on the ionisation constant of the acid (or base). Typical potentiometric acid-base titration curves for a strong acid (and a weak acid) with strong base are given in Fig. 7.3.

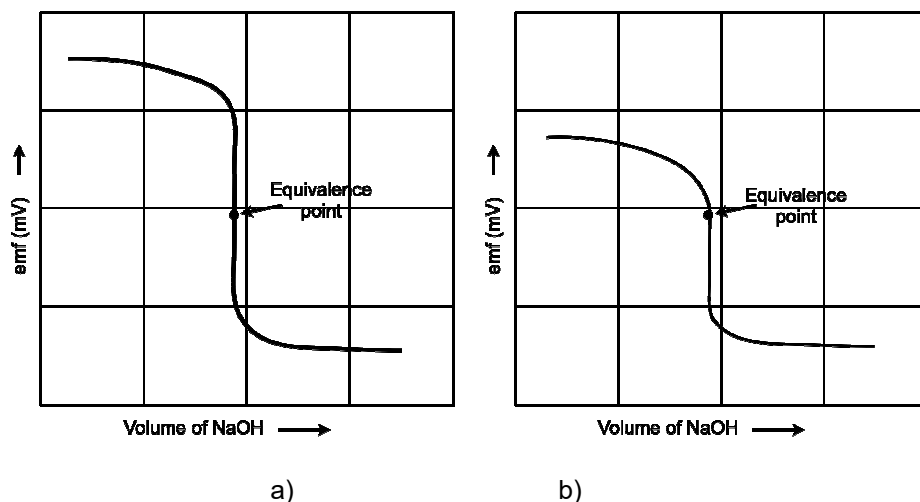


Fig. 7.3: Typical potentiometric acid-base titration curve for a) HCl Vs NaOH and b) CH_3COOH Vs NaOH.

You may note the logarithmic nature of the titration curves. The inflection point (where the slope is maximum negative) is the equivalence point of the titration. It is difficult to locate it visually and is determined by making derivative plots.

The first derivative plot ($\frac{\Delta \text{emf}}{\Delta V}$ versus V) gives the rate of change in the emf

per unit change in the volume as a function of volume of the titrant. A typical first derivative plot for a potentiometric titration curve is given in Fig. 7.4a). The minimum of the plot gives equivalence point. Sometimes the minima in the first derivative plot may be broad and the equivalent point may not be located accurately. In such cases, we draw the second derivative plot ($\frac{\Delta(\Delta \text{emf})}{\Delta(\Delta V)}$ versus V). A typical second derivative plot has also been given in

Fig. 7.4a). In this case, at the equivalence point the value of $\frac{\Delta(\Delta \text{emf})}{\Delta(\Delta V)}$ sharply changes from negative to positive. The point where it meets the x-axis is the equivalence point.

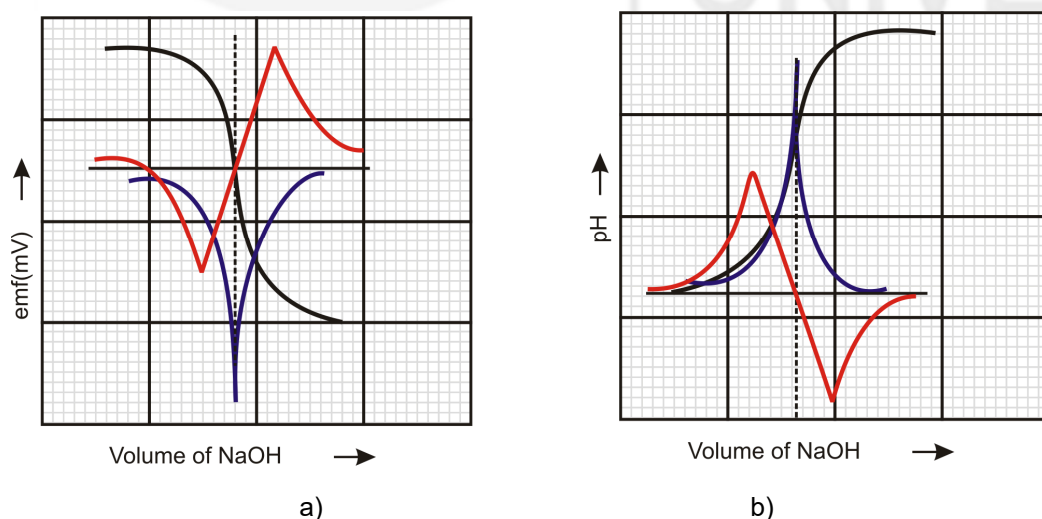


Fig. 7.4: a) Typical potentiometric titration curve (black); corresponding derivative plot (blue) and second derivative plot (red) b) Typical pH titration curve (black); corresponding derivative plot (blue) and second derivative plot (red).

Let us learn how to calculate the data points and to draw the derivative plots?
Let the volume of the titrant added and the observed values of the cell emf be as given in the column 2 and 3 of the Table 7.1.

Calculation of data points for the first derivative plot

The value of V' i.e., the data points to be plotted on the x-axis of the first derivative plot are the average of two consecutive volumes.

$$V'_1 = \frac{(V_1 + V_2)}{2}$$

Table 7.1: Template for calculating data points for derivative plots

S.No	Vol, V	emf, E	V'	$F' = \frac{\Delta \text{emf}}{\Delta v}$	V''	$F'' = \frac{\Delta \text{emf}}{\Delta(\Delta v)}$
1	V_1	E_1				
			$V'_1 = \frac{(V_1 + V_2)}{2}$	$F'_1 = \frac{(E_2 - E_1)}{(V_2 - V_1)}$		
2	V_2	E_2			$V''_1 = \frac{(V'_1 + V'_2)}{2}$	$F''_1 = \frac{(F'_2 - F'_1)}{(V'_2 - V'_1)}$
			$V'_2 = \frac{(V_2 + V_3)}{2}$	$F'_2 = \frac{(E_3 - E_2)}{(V_3 - V_2)}$		
3	V_3	E_3			$V''_1 = \frac{(V'_2 + V'_3)}{2}$	$F''_2 = \frac{(F'_3 - F'_2)}{(V'_3 - V'_2)}$
			$V'_3 = \frac{(V_3 + V_4)}{2}$	$F'_3 = \frac{(E_4 - E_3)}{(V_4 - V_3)}$		
4	V_4	E_4			$V''_3 = \frac{(V'_3 + V'_4)}{2}$	$F''_3 = \frac{(F'_4 - F'_3)}{(V'_4 - V'_3)}$
			$V'_4 = \frac{(V_4 + V_5)}{2}$	$F'_4 = \frac{(E_5 - E_4)}{(V_5 - V_4)}$		
5	V_5	E_5			$V''_4 = \frac{(V'_4 + V'_5)}{2}$	$F''_4 = \frac{(F'_5 - F'_4)}{(V'_5 - V'_4)}$
			$V'_5 = \frac{(V_5 + V_6)}{2}$	$F'_5 = \frac{(E_6 - E_5)}{(V_6 - V_5)}$		
6	V_6	E_6			$V''_5 = \frac{(V'_5 + V'_6)}{2}$	$F''_5 = \frac{(F'_6 - F'_5)}{(V'_6 - V'_5)}$
			$V'_6 = \frac{(V_6 + V_7)}{2}$	$F'_6 = \frac{(E_7 - E_6)}{(V_7 - V_6)}$		
7	V_7	E_7				

The value of F' , i.e., the data points to be plotted on the y-axis of the first derivative plot are the ratio of the difference in emf value and the corresponding difference in the volume of titrant, e.g.,

$$F'_1 = \frac{(E_2 - E_1)}{(V_2 - V_1)}$$

For second derivative plot

The value of V'' , to be plotted on the x-axis are the average of two V' values.

$$V''_1 = \frac{(V'_1 + V'_2)}{2}$$

Similarly, the F'' is the ratio of the difference in F' values and the corresponding difference in the V' values, e.g.,

$$F''_1 = \frac{(F'_2 - F'_1)}{(V'_2 - V'_1)}$$

You may refer to the sample calculation given in Table 7.1, to calculate the data points for plotting derivative plots of your titration curves.

7.3 REQUIREMENTS

Apparatus		Chemicals
Burette(50 cm ³)	1	Hydrochloric acid
Pipette (10 cm ³ and 20 cm ³)	1 each	Sodium hydroxide
Volumetric flask (100 cm ³)	1	Acetic acid
Conical flask (100 cm ³)	1	Oxalic acid
Beakers (50 cm ³)	2	Phenolphthalein
Burette stand and clamp	1	Quinhydrone
Magnetic stirrer with stirring bar	1	Distilled water
Weighing bottle	1	
Analytical balance	1	
Weight box	1	
Funnel	1	
Potentiometer / pH meter	1	
Saturated calomel electrode / silver-silver chloride electrode	1	
Platinum wire	1	
Connecting wires		

Solutions provided

The following solutions would be provided by your counsellor.

1. **Sodium hydroxide solution (~0.1 M):** It is prepared by dissolving about 4 g of sodium hydroxide in about 150-200 cm³ of distilled water and diluting it to 1 litre.
2. **Hydrochloric acid solution (~0.01 M):** It is prepared by adding about 1.0 cm³ of concentrated hydrochloric acid to about 100 cm³ of distilled water and diluting it to 1 litre.
3. **Acetic acid solution (~0.01M):** It is prepared by adding about 0.6 cm³ of glacial acetic to 100 cm³ of distilled water and diluting it to 1 litre.
4. **Phenolphthalein indicator:** It is prepared by dissolving 1 g of the reagent in 100 cm³ of ethanol and adding 100 cm³ of water to it. If a precipitate is formed, it is filtered.

7.4 PROCEDURE

As mentioned in the introduction, you are going to perform two potentiometric titrations in this experiment. The procedure for both of these is same as given below. You will first perform the potentiometric titration of a solution of hydrochloric acid with a solution of NaOH. The procedure consists of the following steps.

- a) Preparation of a standard solution of oxalic acid
- b) Standardisation of sodium hydroxide solution by titrating against standard solution of oxalic acid.
- c) Calibrating the potentiometer (pH /mV meter)
- d) Performing potentiometric titration of the given hydrochloric acid (or acetic acid) solution with standardised solution of sodium hydroxide
- e) Calculation of the concentration of given solution of hydrochloric acid (or acetic acid)

A. Experiment 7.1: Potentiometric titration of HCl with NaOH

Follow the procedure given below in a step wise manner to determine the concentration of given solution of hydrochloric acid potentiometrically.

a) Preparation of 100 cm³ of standard solution of 0.05 M oxalic acid

- i) As you know, to prepare a standard solution we need to first calculate the amount of the substance required. The amount of oxalic acid ($M_m = 126 \text{ g mol}^{-1}$) required to prepare 100 cm³ of 0.05 M solution of oxalic acid would be:

$$\text{mass(ing)} = \frac{M V M_m}{1000} = \frac{0.05 \text{ mol dm}^{-3} \times 100 \text{ cm}^3 \times 126 \text{ g mol}^{-1}}{1000 \text{ cm}^3 \text{ dm}^{-3}} = 0.63 \text{ g}$$

- ii) Weigh about 0.65 g of oxalic acid on a rough weighing balance and transfer it to a clean dry weighing bottle and accurately weigh the weighing bottle with oxalic acid.
- iii) Transfer the oxalic acid to a clean volumetric flask of 100 cm³ capacity through a funnel.
- iv) Weigh the weighing bottle (may contain some oxalic acid) again and find the exact mass of oxalic acid transferred by subtracting this mass from the mass of the weighing bottle plus oxalic acid.
- v) Dissolve oxalic acid in about 30-40 cm³ of distilled water taken in the volumetric flask. Once dissolved, make up the volume up to the mark with distilled water.

b) Standardisation of sodium hydroxide solution by titrating against standard oxalic acid solution

- i) Fill the burette with the given solution of sodium hydroxide using a funnel and mount it on the burette stand. Note the reading on the burette and record it in the Observation Table 7.1 under the initial reading column.
- ii) Carefully pipette out 10 cm³ of the standard solution of oxalic acid and transfer it to a clean 100 cm³ conical flask. Add two to three drops of phenolphthalein indicator to it.
- iii) Titrate the coloured solution with NaOH using constant swirling till a persistent pink colour is obtained. The colour should not fade on shaking the solution. Record the reading in the Observation Table 7.1 under the final reading column.
- iv) Repeat the titration to get at least two concordant readings and record the same in the Observation Table 7.1

c) Calibrating the potentiometer / pH meter

- i) Connect the potentiometer /pH meter to the power mains and allow it to stabilise for about 10-15 minutes.
- ii) Follow the instructions from the operation manual accompanying the instrument and calibrate the instrument. (*Your counsellor will help you in this step*)

d) Performing potentiometric titration of the given solution hydrochloric acid with the standardised solution of sodium hydroxide

- i) Before starting the titration, you should determine the drop volume of your burette. For this adjust the burette (containing NaOH solution) at a certain value (say 0) and note it. Then carefully drain out 100 drops of the solution into a beaker and note the burette reading again. Divide the difference of the two readings by 100 to get the drop volume of the burette.
- ii) Pipette out 20.0 cm³ of the given solution of hydrochloric acid in a 50 cm³ beaker and add a pinch of quinhydrone to get a saturated solution. Dip a platinum electrode into the solution to set up the quinhydrone electrode.
- iii) Take a dip type saturated calomel electrode and dip it into the test solution containing quinhydrone. In case dip type SCE is not available, take saturated solution of KCl in another beaker and insert a saturated calomel electrode into it and connect the two beakers with a salt bridge as shown in Fig. 7.1.

- iv) Drop a magnetic stirrer bar in the beaker containing test solution and keep the beaker on a magnetic stirrer.
- v) Connect the indicator electrode (quinhydrone) and the reference electrode (SCE) to the respective terminals on the calibrated potentiometer as shown in Fig. 7.2.
- vi) Fill the burette with standardised solution of NaOH and mount the burette on an iron stand with a clamp.
- vii) Add a few, say 5 drops of NaOH solution to the test solution, stir and allow it to become still and measure the emf as per the operation manual provided with the instrument. Record your observation in Table 7.2.
- viii) Keep repeating the above step and record your observation in Table 7.2. When you observe larger decrease in the value of emf, decrease the volume of NaOH being added to 1 or 2 drops and continue recording your data.
- ix) Continue the titration for a few readings after the rate of change in emf once again becomes small.
- x) Plot a graph between the measured values of the cell emf and the volume of NaOH added to obtain the titration curve. Locate the equivalence point using derivative plot as explained above.

e) Calculation of the concentration of hydrochloric acid

Convert the volume (in number of drops) of solution of NaOH at the equivalence point to its volume in cm^3 by multiplying with **drop volume** of the burette. Calculate the concentration of the given solution of hydrochloric acid by substituting, the concentration of sodium hydroxide along with the volumes of the sodium hydroxide and hydrochloric acid solutions used, in the molarity equation. Report the result.

Experiment 7.2: Potentiometric titration of CH_3COOH with NaOH

The steps **a to c** of the procedure are the same as given above for the titration of HCl with NaOH. You do not need to repeat them. Follow the procedure given below in a step wise manner to determine the concentration of given solution of acetic acid solution potentiometrically.

d) Performing the potentiometric titration of given solution of acetic acid with standardised solution of sodium hydroxide using emf measurement to determine the equivalence point.

- i) Pipette out 20.0 cm^3 of the given solution of acetic acid in a 100 cm^3 beaker and add a pinch of quinhydrone it to get a saturated solution. Dip a platinum electrode into the solution to set up the quinhydrone electrode.
- ii) Take a dip type saturated calomel electrode and dip it into the test solution containing quinhydrone. In case dip type SCE is not available, take saturated

solution of KCl in another beaker and insert a saturated calomel electrode into it and connect the two beakers with a salt bridge as shown in Fig. 7.1.

- iii) Drop a magnetic stirrer bar in the beaker containing test solution and keep the beaker on a magnetic stirrer.
- iv) Connect the indicator electrode (quinhydrone) and the reference electrode (SCE) to the respective terminals on the calibrated potentiometer as shown in Fig. 7.2.
- v) Fill the burette with standardised solution of NaOH and mount the burette on an iron stand with a clamp.
- vi) Add a few, say 5 drops of NaOH solution to the test solution, stir and allow it to become still and measure the emf as per the operation manual provided with the instrument. Record your observation in Table 7.3.
- vii) Keep repeating the above step and record your observation in Table 7.3. When you observe larger decrease in the value of emf, decrease the volume of NaOH being added to 1 or 2 drops and continue recording your data.
- viii) Continue the titration for a few readings after the rate of change in emf once again becomes small.
- ix) Plot a graph between the measured values of the cell emf and the volume of NaOH added to obtain the titration curve. Locate the equivalence point using derivative plot as explained above.

e) Calculation of the concentration of acetic acid

Convert the volume (in number of drops) of solution of NaOH at the equivalence point to its volume in cm^3 by multiplying with drop volume. Calculate the concentration of the given solution of hydrochloric acid by substituting, the concentration of sodium hydroxide along with the volumes of the sodium hydroxide and acetic acid solutions used, in the molarity equation. Report the result.

7.5 OBSERVATIONS

a) Preparation of 100 cm^3 of standard solution of 0.05 M oxalic acid

Mass of weighing bottle + oxalic acid = $m_1 \text{ g}$ = ...g

Mass of weighing bottle (after transferring oxalic acid) = $m_2 \text{ g}$ = ... g

b) Standardisation of the given solution of sodium hydroxide by titrating against standard solution of oxalic acid.

Volume of standard oxalic acid solution taken in conical flask, $V_o = \dots \text{cm}^3$

Solution in the burette: Sodium hydroxide

Indicator used: Phenolphthalein

Observation Table 7.1: Standardisation of sodium hydroxide

S.No.	Volume of oxalic acid (in cm ³)	Burette reading		Titer value (in cm ³) (Final-initial reading)
		Initial	Final	
1				
2				
3				
Concordant reading				

D. Potentiometric titration of hydrochloric acid (or acetic acid) with sodium hydroxide solution

Record the following readings for the calculation of drop volume of burette

Initial reading of the burette: $R_1 = \dots\dots\dots$

Burette reading after draining 100 drops of solution, $R_2 = \dots\dots\dots$

$$\text{Drop volume} = \frac{(R_2 - R_1)}{100} = \dots\dots\dots \text{cm}^3$$

Volume of hydrochloric acid solution taken in beaker $V_H = \dots \text{cm}^3$

Volume of acetic acid solution taken in beaker $V_A = \dots \text{cm}^3$

Solution in the burette: Sodium hydroxide

Molarity of sodium hydroxide = $M_N = \dots M$

Observation Table 7.2: Potentiometric titration of HCl with NaOH

S.No.	Number of drops of NaOH	E_{cell} (in mV)	S.No.	Number of drops of NaOH added	E_{cell} (in mV)
1			16		
2			17		
3			18		
4			19		
5			20		
6			21		
7			22		
8			23		
9			24		
10			25		
11			26		

12			27		
13			28		
14			29		
15			30		

Observation Table 7.3: Potentiometric titration of acetic acid with NaOH

S. No.	Number of drops of NaOH	E_{cell} (in mV)	S. No.	Number of drops of NaOH added	E_{cell} (in mV)
1			16		
2			17		
3			18		
4			19		
5			20		
6			21		
7			22		
8			23		
9			24		
10			25		
11			26		
12			27		
13			28		
14			29		
15			30		

7.6 CALCULATIONS

Perform the calculations based on the observations recorded above. You need to perform the calculations stepwise. Let us begin with step a.

a) Preparation of 100 cm³ of standard solution of 0.05 M oxalic acid

Molarity of the prepared standard solution of oxalic acid can be calculated as follows:

Mass of weighing bottle + oxalic acid = m_1 g = ...g

Mass of weighing bottle (after transferring oxalic acid) = m_2 g = ... g

Amount of oxalic acid transferred = $m_1 - m_2 = m$ g = ... g

Molar mass (M_m) of oxalic acid = 126 g mol⁻¹

Volume of solution of oxalic acid prepared = 100 cm³

$$\text{Molarity of oxalic acid solution} = M_O = \frac{m \times 1000}{126 \times 100} = \frac{10m}{126} = \dots\dots\dots M$$

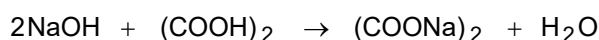
b) Standardisation of the given solution of sodium hydroxide by titrating against standard solution of oxalic acid.

Volume of standard solution of oxalic acid taken in conical flask, $V_O = \dots \text{cm}^3$

Volume of solution of NaOH used (concordant value from Observation Table 7.1) $= V_N = \dots \text{cm}^3$

The concentration of the given solution of sodium hydroxide can be determined as follows:

The reaction involved in the titration:



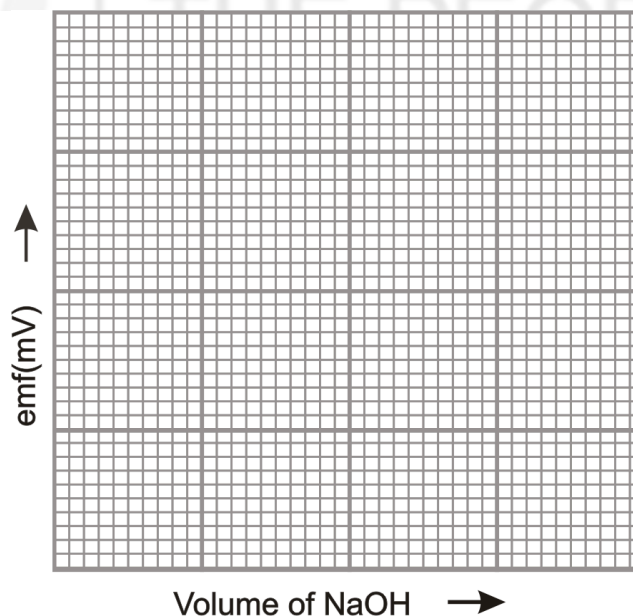
Molarity equation: $M_N V_N = 2M_O V_O$

The molarity of sodium hydroxide = $M_N = \frac{2M_O V_O}{V_N} = \dots\dots M$

Determination of equivalence point

HCl versus NaOH titration

Plot a graph between the measured E_{cell} (in mV; on y-axis) and the volume of solution of NaOH (in number of drops; on x-axis) using the data from Observation Table 7.2. The equivalence point is obtained by drawing first and second derivative plots as explained above. Note the volume of solution of NaOH at the equivalence point. For this, draw a perpendicular on to the x-axis from the equivalence point.

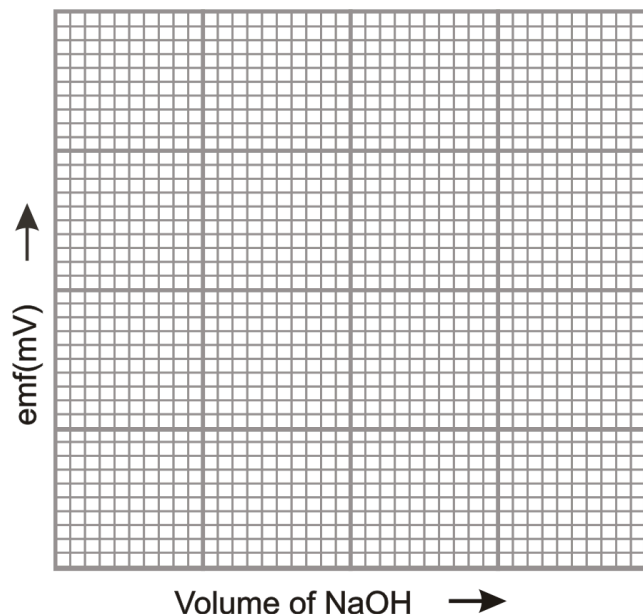


Graph-I: Hydrochloric acid versus NaOH titration

CH₃COOH versus NaOH titration

Plot a graph between the measured E_{cell} (in mV; on y-axis) and the volume of NaOH (in number of drops; on x-axis) using the data from Observation Table 7.3. Determine the equivalence point as given above and record the volume of

solution of NaOH at the equivalence point. For this, draw a perpendicular on to the x-axis from the equivalence point.



Graph-II: Acetic acid versus NaOH titration

Calculation of the concentration of hydrochloric acid

Volume of solution of hydrochloric acid taken in beaker $V_H = \dots \text{cm}^3$

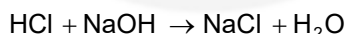
Molarity of solution of sodium hydroxide (obtained from Standardisation), $M_N = \dots M$

Volume of solution of sodium hydroxide used (in number of drops) [from graph-I] = ...

Volume of solution of sodium hydroxide used (in cm^3) =
(Number of drops $\times$ drop volume) = $V_N = \dots \text{cm}^3$

Let molarity of the given solution of hydrochloric acid = M_H

The reaction involved in the titration:



Molarity equation: $M_H V_H = M_N V_N$

The molarity of hydrochloric acid, $M_H = \frac{M_N V_N}{V_H}$

Substituting the values, the molarity of hydrochloric acid = $M_H = \dots M$

Strength of the given solution of hydrochloric acid = molarity $\times$ molar mass

= $M_H \text{ mol dm}^{-3} \times 36.5 \text{ g mol}^{-1} = \dots \text{g dm}^{-3}$

Calculation of the concentration of acetic acid

Volume of the given solution of acetic acid taken in beaker $V_A = \dots \text{cm}^3$

Molarity of the solution of sodium hydroxide (obtained from Standardisation),
= $M_N = \dots M$

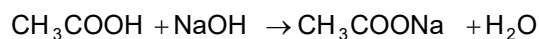
Volume of solution of sodium hydroxide used (in number of drops) [from graph-II] = ...

Volume of solution of sodium hydroxide used (in cm^3) =

$$(\text{Number of drops} \times \text{drop volume}) = V_N = \dots \text{cm}^3$$

Let molarity of the given solution of acetic acid = M_A

The reaction involved in the titration:



Molarity equation: $M_A V_A = M_N V_N$

$$\text{The molarity of acetic acid} = M_A = \frac{M_N V_N}{V_A}$$

Substituting the values, the molarity of acetic acid = $M_A = \dots\dots\dots M$

Strength of the given solution of acetic acid = molarity $\times$ molar mass

$$= M_A \text{ mol dm}^{-3} \times 60 \text{ g mol}^{-1} = \dots\dots\dots \text{g dm}^{-3}$$

7.7 RESULT

1. The strength of given solution of hydrochloric acid determined by potentiometric titration with NaOH is found to be = $\dots\dots\dots \text{g dm}^{-3}$
2. The strength of given solution of acetic acid determined by potentiometric titration with NaOH is found to be = $\dots\dots\dots \text{g dm}^{-3}$

SECTION B



EXPERIMENT 8

SYSTEMATIC QUALITATIVE ANALYSIS OF CARBOXYLIC ACIDS AND PHENOLS AND PREPARATION OF THEIR DERIVATIVES

Structure

8.1	Introduction	Procedure
	Objectives	Observations and Results
8.2	Functional Group Identification	8.4 Experiment 8b: Identification of Phenols and preparation of their derivative
8.3	Experiment 8a: Identification of carboxylic acids and preparation of their derivatives	Functional Group Tests
	Solubility Test	Preparation of Characteristic Derivatives
	Functional Group Test of Carboxylic Acids	Requirement
	Characteristic Derivatives	Procedure
	Requirements	Observation and Result
		8.5 Answers

8.1 INTRODUCTION

Organic compounds are classified into different classes based on the presence of functional groups. In the previous course, BCHCL-134 you have studied elementary analysis of organic compounds, e.g., physical examination, elemental analysis and determination of physical constants etc. In this Experiment and Experiments 9 and 10, you will learn some basic concepts of functional group analysis and perform qualitative tests for identification of some important function groups. Along with this, you will also prepare their derivatives.

Both carboxylic acids and phenols are acidic in nature. Phenols are less **acidic** than **carboxylic acids** and do not react with NaHCO_3 to form water-soluble salts. As a result, phenols are insoluble in 5% NaHCO_3 but most of phenols are soluble in 5% NaOH . In this experiment, our focus will be on qualitative tests used in identification of carboxylic acids and phenols. For the confirmation of the presence of these functional groups, we will also prepare some derivative of carboxylic acid and phenols.

Expected Learning Outcomes

After performing the experiment given, you should be able to:

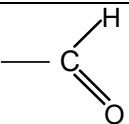
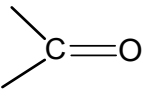
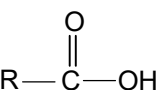
- ❖ explain the meaning of functional group of an organic compound;
- ❖ identify the carboxylic acids by sodium bicarbonate test;
- ❖ identify the phenol by ferric chloride test and Liebermann's test;
- ❖ prepare characteristic derivatives of carboxylic acids; and
- ❖ prepare characteristic derivatives of phenols.

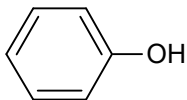
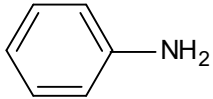
8.2 FUNCTIONAL GROUP IDENTIFICATION

Organic compounds are classified into different classes based on the presence of functional groups. You can recall from your theory course that the functional groups are the sites of chemical reactions. The identification of functional groups depends on their specific features of structure and reactivity.

Somehow identification of functional group depends basically on the correct determination of the elements, which you have studied in BCHCL-133 Course. It is rare for an unknown compound to possess just one functional group. You must always be aware of the possibility that more than one functional group may be present. However, here we will examine only monofunctional compounds. Some common classes of monofunctional organic compounds are listed in Table 8.1

Table 8.1: Common monofunctional organic compounds

Sr.No.	Functional Group	Class	Nature
1)		Aldehydes	Neutral
2)		Ketones	Neutral
3)		Carboxylic acids	Acidic

4)		Phenols	Weakly acidic
5)	-NO ₂	Nitro compounds	Neutral
6)	-CONH ₂	Amides	Neutral
7)		Amines	Neutral

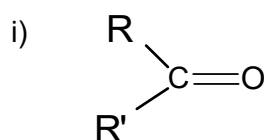
For the identification of the functional group, we generally follow the following steps;

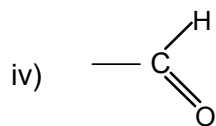
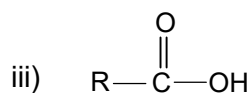
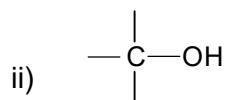
1. Physical examination
2. Elemental analysis
3. Solubility test
4. Identification of functional groups using chemical tests
5. Preparation of derivatives

In your earlier course (BCHCL 134), we have covered steps 1 and 2. In this experiment and in next two experiments (Experiments 9 and 10), our focus will be on the remaining three steps. Our classical scheme for identification of functional group involves performing a number of chemical tests on a substance, each of which is specific for a type of functional group. After confirming the presence of the functional group in an organic compound, we prepare crystalline derivative to identify the actual compound by comparison of melting point with literature value of the compound. The ideal derivative should be able to be simply and quickly prepared with good yield. It should also be easily purified and identified. These should also have sharp and definite melting points preferably between 50°C and 250°C. In this work, you are advised not to jump to premature conclusions about the identity of the compound. Always prepare one derivative and check that its properties agree with those expected values before leaping into the preparation of the second confirmatory derivative. The methods for preparing derivatives for different functional groups will be discussed in this experiment and also in Experiments 9-11.

SAQ 1

Identify the functional group present in the following compounds.





So far in this experiment, we have discussed different steps that are evolved to establish the identity of the unknown compound. Now, we will apply them in identification of carboxylic acids and phenols.

8.3 EXPERIMENT 8a: IDENTIFICATION OF CARBOXYLIC ACIDS AND PREPARATION OF THEIR DERIVATIVES

Before going in detail of chemical tests used for the identification of carboxylic acids and phenols, we would like to discuss solubility test as it plays an important role in the identification of the carboxylic acid functional groups.

8.3.1 Solubility Test

Solubility test of an organic compound in water, dilute acid or dilute base can provide useful, but not definitive, information about the presence or absence of certain functional groups.

Procedure for Solubility Tests

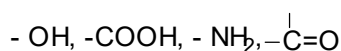
The solubility tests are done at room temperature. Take 100 mg of a solid (finely crushed) or 0.2 cm³ liquid and 3.0 cm³ of solvent in small test tubes. The mixture should be shaken vigorously. It is recommended that the solubility tests to be done in the order presented below:

Solubility in Water

Weight 100 mg of the finely crushed solid or measure 0.2 cm³ of liquid with the help of graduated pipette or burette and treat it with successive 1.0 cm³ portion of distilled water; shake vigorously after each addition until 3.0 cm³ have been added. If the compound does not dissolved completely in 3.0 cm³ of water, it may be regarded as insoluble in water.

Now test the contents of the test tube with pH paper. This test can be done by taking one drop of solution with the help of glass rod and touching it with pH paper.

The solubility of the compound in distilled water suggests that the compound is a low molecular weight organic compound having 4-5 carbon atoms, which contain a polar group such as



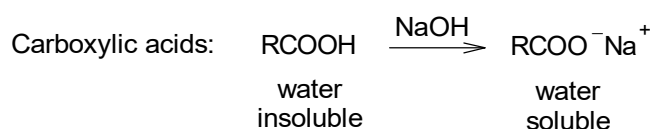
If higher molecular weight compound soluble in water, it must be polyfunctional, for example carbohydrates. On the other hand, alkanes, alkenes, alkynes and alkyl halides are water-insoluble.

If the organic compound is water soluble and gives a positive acidic test with pH paper, it is likely to be a low molecular weight carboxylic acid such as acetic acid. If compound gives positive basic test with pH paper, the compound is a low molecular weight organic base such as diethylamine. A neutral solution suggests the presence of a neutral polar compound such as ethanol or acetone.

Solubility in 5% Sodium Hydroxide

If the compound is insoluble in distilled water, it should be similarly tested for solubility in 5% aqueous sodium hydroxide solution. This solubility indicates the presence of a carboxylic acid, sulphonic acid, or phenol. Because they are converted into their water – soluble sodium salts.

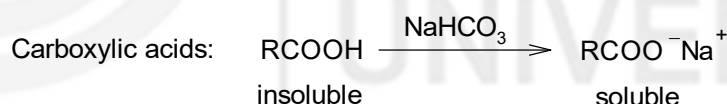
Highly acidic phenols are soluble in 5% sodium bicarbonate.



NaOH soluble organic compound should then be tested for solubility in the weaker base, 5% NaHCO₃ solution, which may permit distinction between carboxylic and phenolic functional groups.

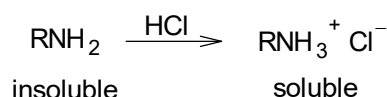
Solubility in 5% Sodium hydrogencarbonate (Sodium bicarbonate) Solution

Similar to the procedure adopted for the solubility of water, the solubility of the compound is checked with 5% aqueous solution of sodium hydrogencarbonate. If it is soluble, the presence of a carboxylic acid group may be tentatively concluded, owing to the formation of the water-soluble sodium salt. Phenols are insoluble in sodium hydrogencarbonate.



Solubility in 5% Hydrochloric Acid

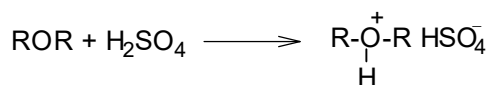
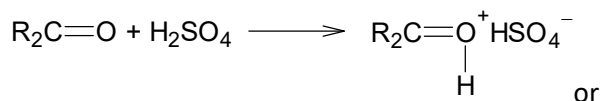
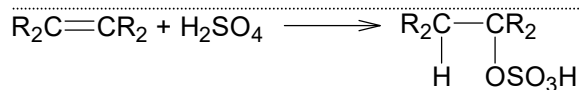
The solubility in 5% aqueous hydrochloric acid is evidence for an amino compound or a heteroaromatic base.



Any unknown insoluble in all the reagents so far should also be checked for solubility in concentrated sulphuric acid.

Solubility in Concentrated Sulphuric Acid

Take 3 cm³ of concentrated sulphuric acid with the help of graduated pipette in a dry test tube and add 100 mg of solid or 0.2 cm³ of liquid. The solubility of organic compound in sulphuric acid indicates the presence of oxygenated and unsaturated aliphatic compound.



The general solubility behaviour of common classes of organic compound is shown schematically in Fig. 8.1.

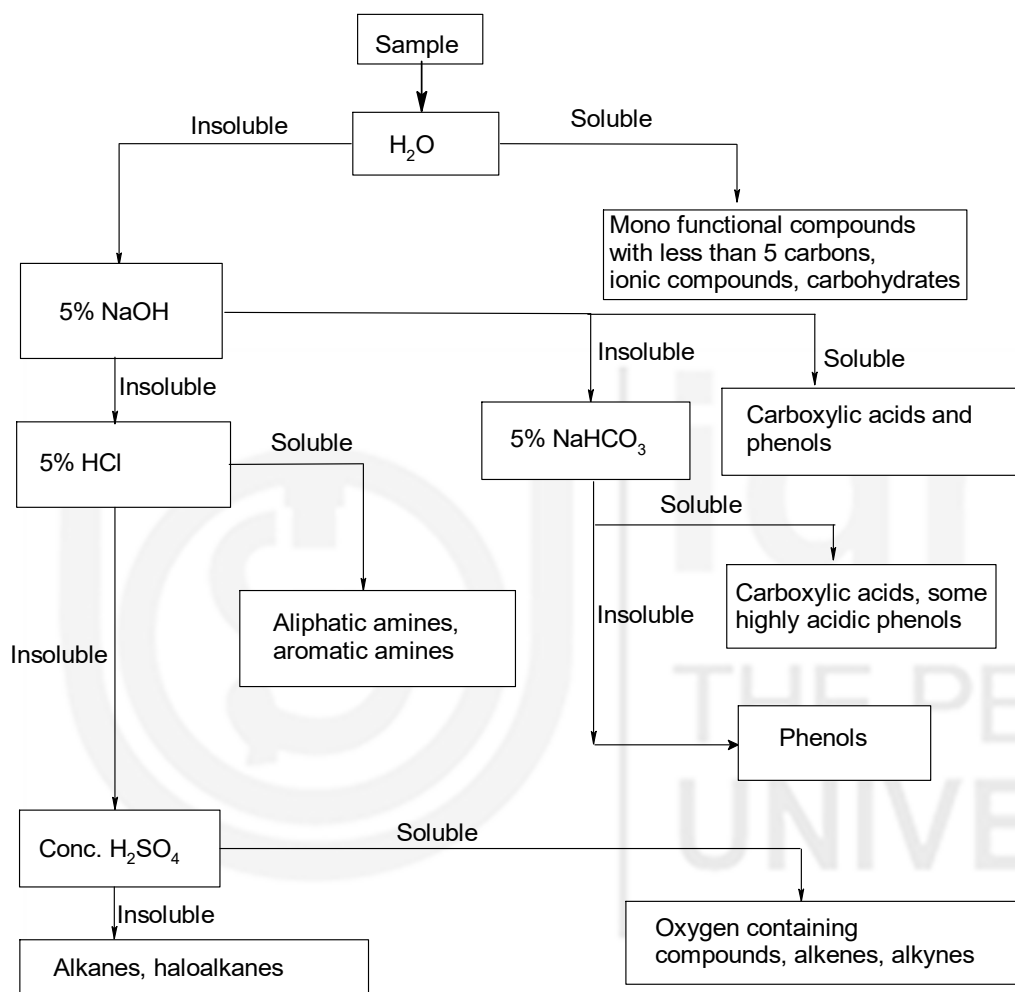


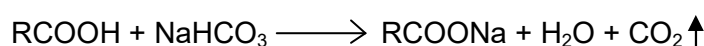
Fig.8.1: Solubility behaviour of common classes of organic compounds.

8.3.2 Functional Group Test for Carboxylic Acids

Carboxylic acids are represented by general formula $RCOOH$. The $-COOH$ group is known as carboxylic group. The presence of carboxylic group in the compound is ascertained by following test:

Sodium Bicarbonate Test

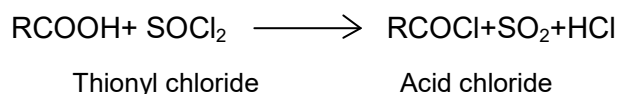
One of the best tests for the carboxylic group is solubility in basic solution. Carboxylic acids liberate carbon dioxide from sodium bicarbonate.



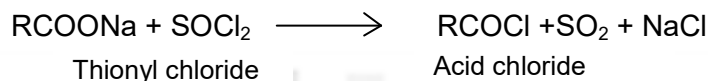
Take 0.2 g of the unknown compound in a test tube and add 1 cm³ of 5% aqueous sodium bicarbonate. Vigorous evolution of carbon dioxide with effervescence indicates the presence of carboxylic group.

8.3.3 Characteristic Derivatives

Common derivatives prepared for identification of carboxylic acids are: amides, anilides and *p*-toluidides. Amides, anilides and *p*-toluidides are prepared from the corresponding acid chloride by treatment with ammonia, aniline or *p*-toluidine, respectively. It is considered that the method for the preparation of anilides and *p*-toluidides has advantage over amide. This is because amides are more soluble in water and as a result are harder to isolate. The acid chloride is prepared from the acid or its salt, and thionyl chloride.



or



Be very careful while adding conc. NH₄OH, the reaction is quite vigorous.

Acid chloride can be used to make the amide, anilides or *p*-toluidides. Therefore, let us first study the method for preparation of acid chloride. Then, we will learn the method for preparation derivative of carboxylic acids.

Preparation of Acid Chloride

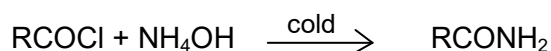
Take the sample of carboxylic acid (1 g), thionyl chloride (2 cm³) and dimethylformamide (DMF) (5 drops) in a small round bottom flask. Attach a reflux condenser and reflux for about 30 minutes. Precipitate of acid chloride will appear at the bottom of the flask. This mixture, containing acid chloride, can be used to prepare amide, anilide or *p*-toluidide derivative as described below:

Procedure for Preparing Derivatives

Common derivatives used for identification of carboxylic acids are Amides, *p*-Toluidides and Anilides Derivatives. Let us discuss the method of preparation for these derivatives individually.

i) Amide Derivative

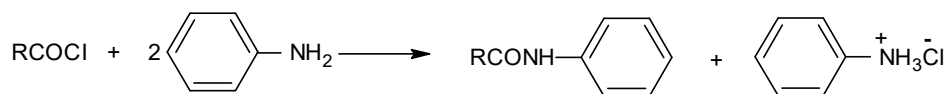
To about 2 gm of the above mixture, containing acid chloride, and add 15 cm³ of ice-cold conc. NH₄OH. A vigorous reaction takes place. Filter off the solid formed as the result of the reaction. Recrystallise it from water or aqueous ethanol. The reaction involved in this process is:



ii) *p*-Toluidides and Anilides Derivatives

Take about 2 g of the crude acid chloride in a 100 cm³ conical flask and dissolve it in 5 cm³ of acetone. To this add 1 g *p*-toluidine (dissolved in

acetone). Shake the mixture for few minutes and add 50 cm³ of NaOH to the flask. Filter off the solid *p*-toluidide which formed during the reaction. Wash the *p*-toluidide with water and recrystallised from ethanol. The reaction involved in this process is:



If you want to prepare anilide, use aniline in place of *p*-toluidine and you will get anilides.

After drying the derivative, determine its M.P and compare it with theoretical value.

SAQ 2

Fill in the blanks.

- Carboxylic acids liberate from sodium bicarbonate.
- Reaction of acid chloride with ammonia yields
- Reaction of carboxylic acid with yields anilides.

SAQ 3

Complete the following reactions

- $\text{RCOOH} + \text{NaHCO}_3 \longrightarrow \dots + \dots + \dots$
- $\text{RCOOH} + \text{SOCl}_2 \longrightarrow \dots + \dots + \dots$
- $\text{RCOCl} + \text{NH}_4\text{OH} \longrightarrow \dots$

8.4.3 Requirements

S. No.	Apparatus	S. No.	Chemicals
1.	Beakers	2.	NaOH
2.	Conical flask	3.	Acetone
3.	Droppers	4.	Carboxylic acid
4.	M.P. apparatus	5.	Ethanol
5.	Reflux condenser	6.	conc. H ₂ SO ₄
6.	Round bottom flask	7.	HCl
7.	Test tubes	8.	conc. NH ₄ OH
8.	Thermometers	9.	<i>p</i> -Toluidine
9.	Water bath	10.	Pyridine
		11.	Sodium bicarbonate
10.	pH paper	12.	Thionyl chloride
		13.	2,4-Dimethylformamide (DMF)
			2-3 Samples of Carboxylic acid

8.3.4 Procedure

1. Collect sample(s) of carboxylic acid from your counsellor and carry out physical examination of the sample as discussed in BCHCL 134 course.
2. Carry out solubility test as discussed in Sec. 8.3.1. If your compound is soluble in water, check the pH of solution using pH paper.
3. Carry out functional group test (class determination test) as discussed in Sec. 8.3.2.
4. Prepare at least one derivative using procedure discussed in 8.3.3
5. Dry the prepared derivative and take the m.p. of both derivative and sample if it is solid. Predict the actual compound by comparison of melting point of sample and its derivative with literature values. Use appendix A for this purpose.

8.3.6 Observations and Result

1. Physical Examination

- a) Physical state :
- b) Colour :
- c) Odour :
- d) Ignition test :

Comment: This suggests that the unknown 'A' is either aromatic or aliphatic compound

2. Elemental Analysis: Not necessary at this point

3. Solubility test

H ₂ O	5% NaOH	5% NaHCO ₃	5% HCl	Conc. H ₂ SO ₄	Expected Class

4. Test with pH paper :

5. Melting point/Boiling point observed :

5. Class determination :

Sodium bicarbonate Test

6. Preparation of derivatives

(a) Derivative chosen:

M.P. observed

(b) Derivative chosen

M.P. observed

7. Examination of literature

Possible compound	M.P. in literature	Derivative(s)/ M.P. in literature	M.P. of Sample	M.P. of Derivative(s)

8. The unknown compound is

9. The structure of this compound is.....

8.4 EXPERIMENT 8B: IDENTIFICATION OF PHENOLS AND PREPARATION OF THEIR DERIVATIVES

The aromatic compounds in which hydroxyl (OH) group is directly attached to benzene ring are called phenols. Phenols are mildly acidic. These require careful handling, especially in laboratories, because these can cause chemical burns. There are few laboratory tests which confirm the presence of phenolic group in organic compounds.

In this section, we shall study the method for carrying out functional group tests of phenols and preparation of their characteristic derivatives.

8.4.1 Functional Group Test

There are following three main functional group tests for identification of phenols.

- i) Ferric Chloride Test
- ii) Liebermann's Test
- iii) Ceric Ammonium Nitrate Test

Following apparatus and chemicals are required for these tests;

i) Ferric Chloride Test

Most phenols react with ferric chloride and give colour. Some phenols, that do not give colour in aqueous or alcoholic solution, but they do so in chloroform especially after addition of a drop of pyridine. Some phenols do not give colour at all. So a negative test must not be taken as significant without supporting further information.

Procedure

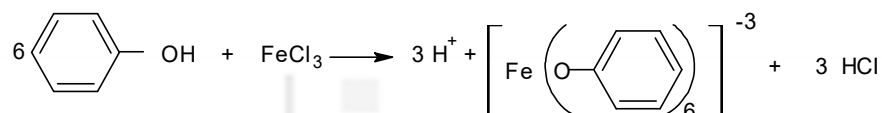
Dissolve 0.5 g of the unknown compound in 1-2 cm³ of water (or a mixture of water and 95% ethanol if the compound is not water soluble) and add a few drops of very dilute (1%) ferric chloride solution. Wide range of colours given below, show the presence of phenolic -OH.

Compound	Colour
Phenol, o-Cresol	Violet
<i>p</i> -Cresol, quinol	Blue
<i>m</i> -Cresol, 1-Naphthol(alcoholic)	Blue-violet
Resorcinol	--
1- Naphthol	Pink
2- Naphthol (alcoholic)	Green

Following compounds do not respond with ferric chloride test,

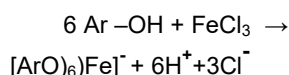
- Picric acid
- Naphtholsulphonic acid

The reaction involved in this test is,



Enols ($\text{R}'\text{C}=\text{CHR}'$) also give a wide range of colour with FeCl_3 .

The phenol complexes are probably coordination compounds in which iron is hexavalent.



There are mainly to functional group tests for identification of phenols which are given below:

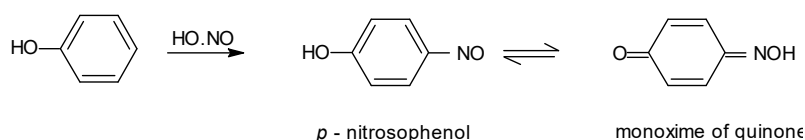
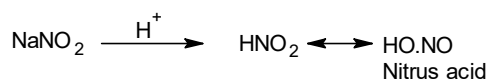
ii) Liebermann's Test

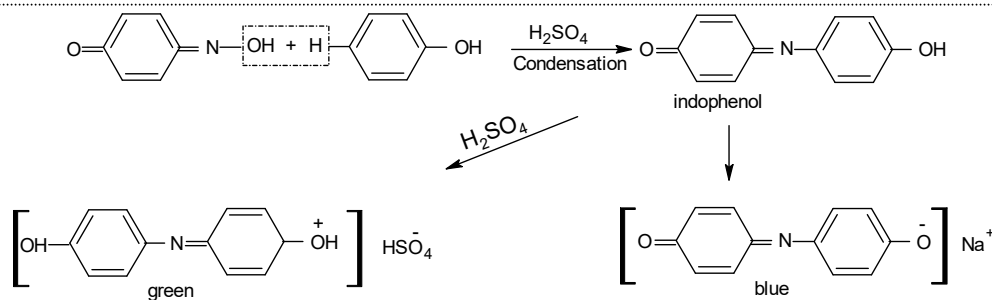
Phenols in which *para* position is free, give positive Liebermann's test, although few exception are noted. For example, *p*-substituted phenols and nitrophenols do not respond to Liebermann's test. Dihydroxyphenols, except resorcinol, do not give satisfactory result with this test.

Procedure

Take few crystals of sodium nitrite and compound (0.5 g) in a dry test tube. Heat the mixture gently for about 30 seconds, allow it to cool and add 0.5 cm³ of conc. sulphuric acid. On shaking the contents a deep green or blue colour develops. Dilute the content with water, the colour changes to red. Now add excess of dilute sodium hydroxide solution, the green or blue again reappears. Colour formation is observed due to the production of salt of indophenols.

The reactions of the test are:





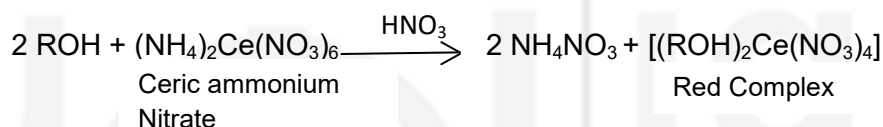
iii) Ceric Ammonium Nitrate Test

Ceric ammonium nitrate can also be used as a qualitative test for phenols. You carry out the test at room temperature because hot solutions of the reagent oxidise many of organic compounds.

Procedure

Prepare the solution of the unknown compound (0.2 g or 1 cm^3) in water, (or dioxane for water insoluble compounds) add few drops of ceric ammonium nitrate. A red colour is obtained. This indicates the presence of primary, secondary and tertiary alcohols. Alcohols replace nitrate ions in complex cerate anions, resulting in a change from a yellow to red solution.

The preliminary reaction for phenol/alcohols is,



8.4.2 Preparation of Characteristic Derivatives

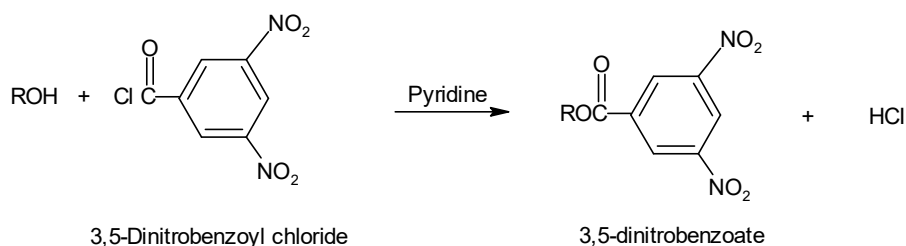
Some common derivatives which are used to identify the phenols are:

- i) 3,5- Dinitrobenzoate Derivatives
- ii) 1-Naphthyl Urethane Derivative

Let us discuss each of them separately

i) 3,5- Dinitrobenzoate Derivatives

The reaction between 3,5-dinitrobenzoyl chloride and alcohol gives the corresponding ester (3,5-dinitrobenzoate). 3,5-Dinitrobenzoate esters are suitable derivatives for both alcohols and phenols. The reaction involved in this preparation is as under:



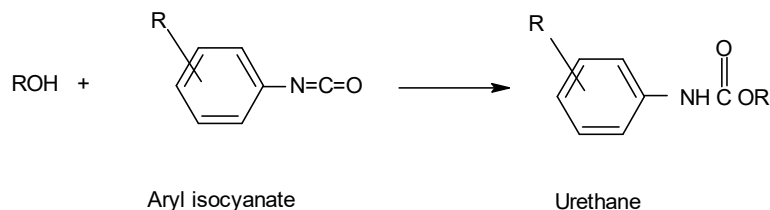
Procedure

Dissolve the alcohol (2 cm^3) in dry pyridine (5 cm^3) and add 3,5-dinitrobenzoyl chloride (1.2 g) in a 100 cm^3 round-bottomed flask. Reflux

the reaction mixture for about 30 minutes and pour it into 40 cm³ of hydrochloric acid. Separate the solid or oily product and stir with 15 cm³ sodium carbonate solution (1M) to remove any 3,5-dinitrobenzoic acid formed. Filter the solid and recrystallised from petroleum (60-80° C), ethanol or aqueous ethanol.

ii) 1-Naphthyl Urethane Derivative

This derivative is also suitable for both alcohols and phenols. When aryl substituted isocyanate, ArNC=O, react with alcohols, it gives a urethane.



A major side reaction is that of water with isocyanate. To avoid the side reaction, take precautions to ensure that the alcohol is **anhydrous**.

Procedure

Place the phenol (0.5 g), dry pyridine (1 cm³) and 1-naphthylurea (0.5 cm³) in a dry test tube. Shake the mixture for few minutes. If no precipitate forms, warm gently on a water bath for 5 minutes and then cool the mixture in ice. Filter off the solid product. Recrystallise the crude derivative from petroleum ether (40-60°C). (Remove 1-naphthylurea by filtration which is insoluble in petroleum).

SAQ 4

Which of the following would give colour with FeCl₃?

- i) *p*-cresol
 - ii) Phenol
 - iii) Resorcinol
 - iv) 2-Naphthol (alcoholic)
-

SAQ 5

Which one of the following compounds does not show colour with FeCl₃

- i) Picric acid
 - ii) *o*-cresol
 - iii) Quinol
 - iv) 2-Naphthol (alcoholic)
-

SAQ 6

Give the three main functional group tests for identification of phenols.

8.4.3 Requirements

S. No.	Apparatus	S. No.	Chemicals
1.	Beakers	2	1. conc. NH_4OH
2.	Conical flask		2. pH Paper
3.	Droppers	1	3. Carboxylic acid
			4. Ethanol
4.	M.P. Apparatus	1	5. Pyridine
5.	Reflux condenser	1	6. Acetone
6.	Round bottom flask	1	7. Sodium nitrite
7.	Test tubes	3	8. 3,5-Dinitrobenzoyl chloride
8.	Thermometers	1	9. Ceric ammonium nitrate
9.	Water bath	1	10. H_2SO_4
			11. HCl
			12. NaOH
			13. Ferric chloride
			14. Sodium carbonate
			15. petroleum ether (40-60°C)
			16. 1-naphthylurea
			17. Samples of Phenols

8.4.4 Procedure

1. Collect sample(s) of phenol from your counsellor and carry out physical examination of the sample as discussed in BCHCL 134 course.
2. Carry out solubility test as discussed in Sec. 8.3.1. Check the pH of water solution using pH paper.
3. Carry out class determination test as discussed in Sec. 8.4.1.
4. Prepare at least one derivative using procedure discussed in 8.4.2
5. Dry the prepared derivative and take the m.p. of both the derivative and sample if your sample is solid. Predict the actual compound by comparison of melting point of sample and its derivative with literature values.

8.4.5 Observations and Result

1. Physical Examination

- a) Physical state :
 b) Colour :
 c) Odour :
 d) Ignition test :

Comment: This suggests that the unknown 'A' is aromatic/aliphatic compound.

2. Elemental Analysis: Not necessary at this point.

3. Solubility test

H ₂ O	5% NaOH	5% NaHCO ₃	5% HCl	Conc. H ₂ SO ₄	Expected Class

4. Test with pH paper :

5. Melting point/Boiling point observed :

5. Class determination

Ferric Chloride Test

Liebermann's Test

Ceric Ammonium Nitrate Test

6. Preparation of derivatives

(a) Derivative chosen

M.P. observed

M.P. in literature

(b) Derivative chosen

M.P. observed

M.P. in literature

7. Examination of literature

Possible compound..... M.P..... Derivatives

8. The unknown compound is

9. The structure of this compound is.....

8.5 ANSWERS

Self Assessment Questions

1.
 - i) Ketones
 - ii) Alcohols
 - iii) Carboxylic Acids
 - iv) Aldehydes
2.
 - i) Carbon dioxide
 - ii) Amide
 - iii) Aniline
3.
 - i) $\text{RCOONa} + \text{H}_2\text{O} + \text{CO}_2\uparrow$
 - ii) $\text{RCOCl} + \text{SO}_2 + \text{HCl}$
 - iii) RCONH_2
4. All four
5. i)
6.
 - i) Ferric Chloride Test
 - ii) Liebermann's Test
 - iii) Ceric Ammonium Nitrate Test

SYSTEMATIC QUALITATIVE ANALYSIS OF ALDEHYDES AND KETONES AND PREPARATION OF THEIR DERIVATIVES

Structure

9.1	Introduction	Procedure
	Expected Learning Outcomes	Observation and Result
9.2	Aldehydes and Ketones	9.3 Answers
	Functional Group Tests	
	Preparation of Characteristic Derivatives	
	Requirement	

9.1 INTRODUCTION

In the last experiment, we have learnt some basic concepts of functional group chemistry and performed qualitative tests for identification of carboxylic acids and phenols and methods of preparation of their derivatives. In this experiment, you will carry out tests for the identification of aldehydes and ketones using Brady's test, Tollen's test, Schiff test and Iodoform test. You will also perform tests to differentiate these two categories of compounds. Once your functional group has been determined, you will prepare a derivative of your compound to establish actual identity of your given sample.

Expected Learning Outcomes

After performing the experiment, you should be able to:

- ❖ identify the aldehydes and ketones by Brady's test, Tollen's test and Schiff test.
- ❖ prepare characteristic derivatives of aldehydes and ketones.

9.2 EXPERIMENT 9: IDENTIFICATION OF ALDEHYDES AND KETONES AND PREPARATION OF THEIR DERIVATIVES

Both aldehydes and ketones have carbonyl group ($>\text{C}=\text{O}$). You have studied the reactions of carbonyl group in organic chemistry course (BCHCT 133). In this experiment you will perform the tests for detecting aldehydes and ketones. In the last section, you will learn the methods of preparation of derivatives of aldehydes and ketones.

9.2.1 Functional Group Test

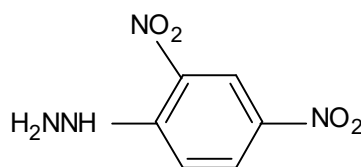
There are four main following functional group tests for identification of aldehyde and ketones. They are:

- i) Brady's Test
- ii) Tollen's Test
- iii) Schiff Test
- iv) Iodoform Test

Let us discuss each of these separately.

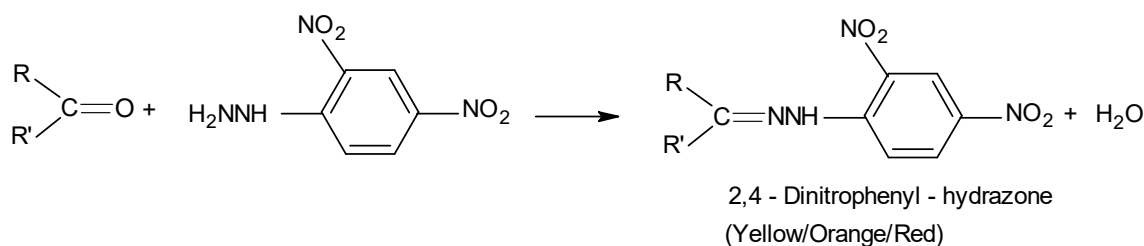
i) Brady's Test

2,4-Dinitrophenylhydrazine (2,4-DNP) is known as Brady's reagent. It is a red-orange solid, usually supplied wet to reduce the risk of explosion. The structure of 2,4-Dinitrophenylhydrazine is given below.



2,4-Dinitrophenylhydrazine derivatives offer a convenient way of separating the components of a mixture of aldehydes and ketones.

The carbonyl compounds (aldehydes/ketones) react with 2,4-dinitrophenylhydrazine (DNP) and give crystalline 2,4-dinitrophenylhydrazone product having orange/ yellow/ red colour. The reaction involved in the Brady's test is given:



This test is carried out as given below:

Procedure

Take 0.5 g of the unknown compound in a test tube and dissolve it in few drops of water or ethanol. Add 1 cm³ of Brady's reagent. Heat the reaction

mixture on water bath for few minutes and then cool it in ice. The appearance of yellow/orange/red crystalline precipitate indicates the presence of aldehydes or ketones.

The colour of the precipitate gives some idea of the type of carbonyl compounds.

Colour of Precipitate	Compound
Yellow	Saturated carbonyl compounds
Orange	α , β -Unsaturated carbonyl compounds
Red	Aromatic carbonyl compounds

SAQ 1

Write the structural formula of 2,4-Dinitrophenylhydrazine.

ii) Tollen's Test

If Brady's test is positive, then aldehydes and ketones are differentiated through Tollen's test. A positive Tollen's test is indicated by the or sometimes precipitation of elemental silver, often producing a characteristic "silver mirror" on the inner surface of the reaction vessel.

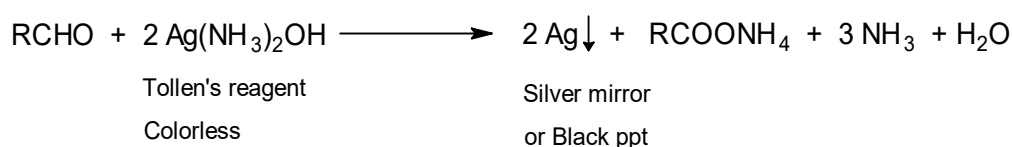
Tollens' reagent is used to determine the presence of aldehydes in organic compounds. The reagent consists of a solution of silver nitrate, ammonia and sodium hydroxide (to maintain a basic pH of the reagent solution). It was named after its discoverer, the German chemist Bernhard Tollens. A positive Tollens test indicates the presence of an aldehyde. Normally, ketones do not give Tollen's test but there are few exceptions, for example acetophenone and cyclohexanone. Some easily oxidisable compounds (e.g., certain phenols, 2-naphthol; and amines) also give positive Tollen's test but these compounds do not give positive test with DNP.

Silver ammonium ion $[\text{Ag}(\text{NH}_3)_2]^+$ is present in Tollen's reagent. In this test, aldehyde is oxidised to carboxylic acid and silver ion reduced to free silver.

Procedure

Place the unknown compound (2 drops of liquid or 0.05 g of solid) and add 1 cm^3 of freshly prepared Tollen's reagent in a clean test tube. Shake the test tube and place it in the stand for about 10 minutes. If black precipitate or silver mirror on the wall of test tube is not appeared, warm the test tube for few minutes on a water bath. Formation of black precipitate of silver or silver mirror indicates the presence of aldehydic group.

The reaction can be given as:

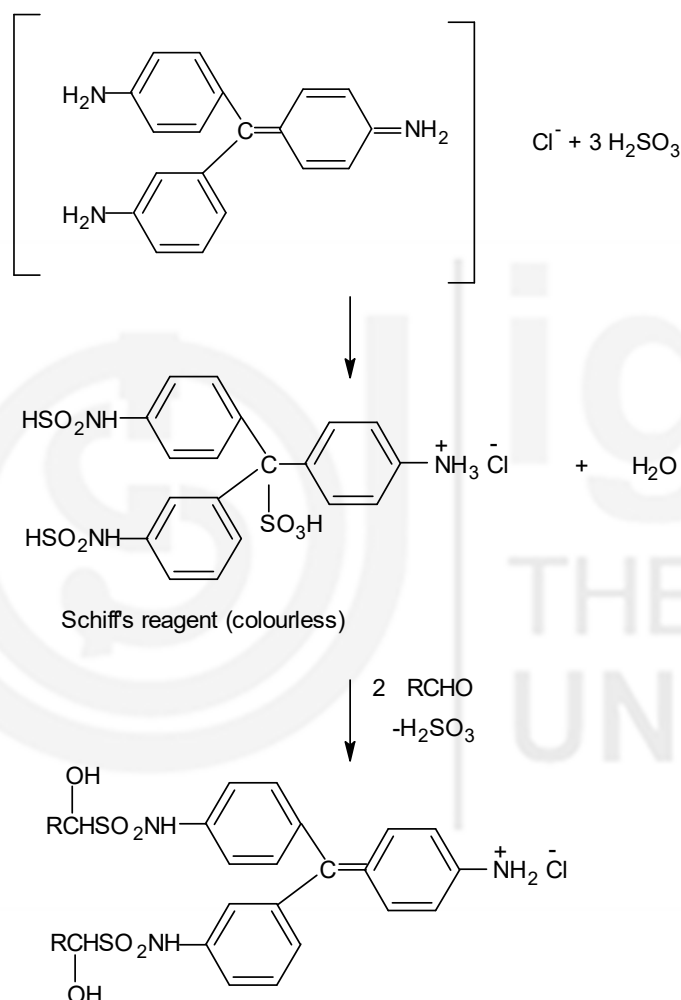


SAQ 2

Name the chemist who discovered the Tollens Test.

iii) Schiff's Test

Another method for distinguishing between aldehyde and ketone is Schiff Test. Aldehydes give positive Schiff's test whereas ketones do not react with Schiff's reagent. In this test, an aldehyde reacts with Schiff's reagent to form a characteristic magenta colour. Some aromatic aldehydes (e.g., vanillin), give a negative result with the Schiff's reagent. Methyl ketones ($\text{CH}_3\text{CO}-$) may restore the colour of Schiff's reagent very slowly. Your test tube should be free from alkali and the salts of weak acids because these also redden the Schiff's reagent like an aldehyde. The reaction sequence of this is:



Perform this test as given below:

Procedure

Take 2 cm^3 of unknown compound or 2 cm^3 of aqueous or alcoholic solution of the unknown compound in a test tube. Then add 2 cm^3 of Schiff's reagent in the test tube and shake it for 2 minutes. **Do not warm or heat** the Schiff's reagent because pink colour develops on heating even in the absence of aldehyde. The appearance of wine-red or purple colour indicates the presence of an aldehyde group.

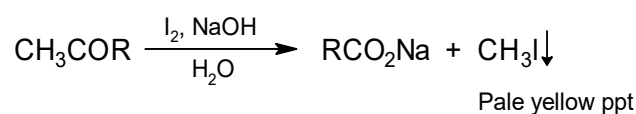
SAQ 3

What are the compounds involved in the preparation of Schiff 's reagent?

iv) Iodoform Test

Iodoform test is useful for the identification of methyl ketones (CH_3COR) and secondary methyl carbinols $\text{CH}_3\text{CH}(\text{OH})\text{R}$. Since this test is positive for $\text{CH}_3\text{CH}(\text{OH})$ - groups, it is advised that you should perform iodoform test after confirming the presence of carbonyl group. Ethanol, acetaldehyde (ethanol), CH_2ICO - also gives positive iodoform test. The test is negative for acetic acid and some other similar compounds.

Reaction involved in the test is given by,



Carry out the test as given below:

Procedure

Dissolve 0.5 cm^3 of the unknown liquid or 0.2 g of the solid in water (3 cm^3) or aqueous dioxane (2 cm^3 water + 2 cm^3 dioxane) in a boiling tube. Add iodine-potassium iodide solution (1 cm^3) and then few drops of 3 M sodium hydroxide solution with shaking until the brown colouration vanishes. A positive test is indicated by immediate formation of pale yellow precipitate of iodomethane (iodoform), melting point. 119°C , without heating.

SAQ 4

Write the reaction involved in the Iodoform test.

SAQ 5

Which of the following compounds will give a positive haloform test?

- a) $\text{C}_6\text{H}_5\text{COCH}_3$; b) CH_3COOH ; c) CH_3CHO ;
d) CH_2ICOR ; e) $\text{CH}_3\text{COCH}_2\text{COOR}$
-

9.2.2 Preparation Characteristic Derivatives

For the preparation of derivatives, carbonyl group of aldehydes and ketones are converted into another groups such as $> \text{C} = \text{NNHR}$, oximes, etc. Some important derivatives which help in the identification of aldehyde and ketones are:

- i) 2,4-Dinitrophenylhydrazone

ii) Semicarbazone

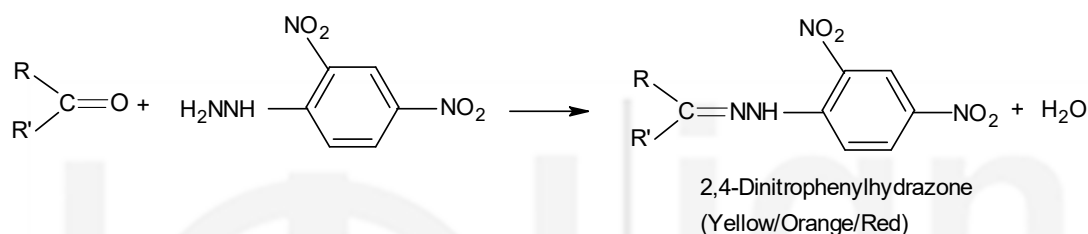
iii) Oximes

Methods for the preparation of some common derivatives are given below this section.

i) 2,4-Dinitrophenylhydrazone

Procedure

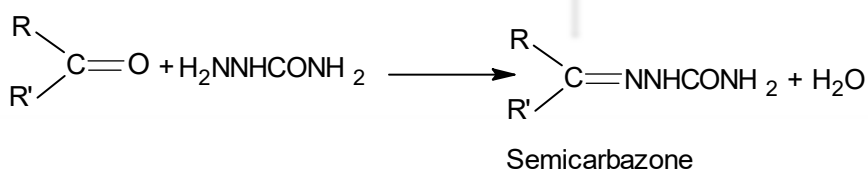
Dissolve 0.5 g of the unknown carbonyl compound in a small volume of ethanol and add 2-3 cm³ of the 2,4-dinitrophenylhydrazine reagent. Heat the mixture on water bath for 15-20 minutes, if no solid separates immediately, cool it and if a precipitate still does not form, add water dropwise until precipitate forms. Filter off the resulting solid, wash it with aqueous methanol (equal volumes of water) and methanol and recrystallise the solid from ethanol or ethyl acetate. Take the melting point. Reaction involve in the process is given below:



ii) Semicarbazone

Procedure

Dissolve the semicarbozide hydrochloride (0.5 g) and sodium acetate (0.8 g) in 5 cm³ of water in a test tube and then add the carbonyl compound (0.5 g). Add minimum amount of ethanol dropwise to get a clear solution if the solution is not clear. Heat the mixture for 10-20 minutes on a water bath, cool in the ice and filter. Recrystallise the product from ethanol, aqueous ethanol, water or benzene.

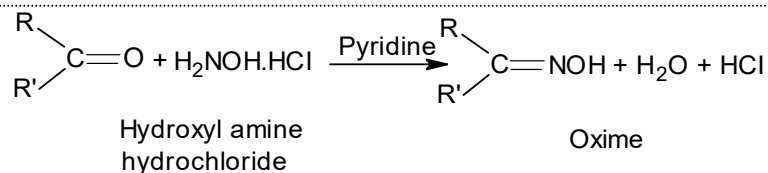


iii) Oximes

This is used for higher molecular weight aldehydes and ketones.

Procedure

Place a mixture of 0.5 g of carbonyl compound, 0.5 g of hydroxylamine hydrochloride, 3 cm³ of pyridine and 3 cm³ of absolute ethanol in a small round bottom flask. Fit reflux condenser to the round bottom flask and reflux the mixture for 2 hours on water bath. Evaporate the recrystallised the residue from ethanol or aqueous ethanol. The reaction involved in the preparation of oxime is given below:



SAQ 6

What are the main derivatives used to for identification of aldehyde an ketones?

SAQ 7

Write the chemical formula of the following compounds.

- i) 2,4-Dinitrophenylhydrazone
- ii) Semicarbazone
- iii) Oximes

9.2.3 Requirements

S. No.	Apparatus	Qty.	S. No.	Chemicals
1	Beakers	2	1	Alcohol
2	Boiling tube	1	2	2,4-Dinitrophenylhydrazine (2,4-DNP)
3	Conical flask	1	3	Dioxane
4	Droppers	1	4	Ethanol
5	M.P. Apparatus	1	5	Hydroxylamine hydrochloride
6	Reflux condenser	1	6	Iodine- potassium iodide solution
7	Round bottom flask	1	7	Methanol
8	Test tubes	3	8	Pyridine
9	Thermometers	1	9	Schiff 's reagent
10	Water bath	1	10	Semicarbazide hydrochloride
			11	Sodium acetate
			12	Tollen's reagent
			13	Samples of aldehydes and ketones

Solution Provided

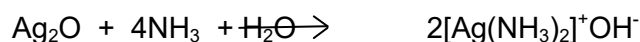
Brady's reagent: Dissolve 1 g of DNP in 5 cm³ concentrated sulphuric acid. Add this solution slowly with shaking and cooling to a mixture of water (7 cm³) and ethanol (25 cm³). Filter it to remove any suspended solid and store as a stock solution.

Tollen's reagent:

Solution A: Dissolve silver nitrate (2.5 g) in distilled water (40 cm³).

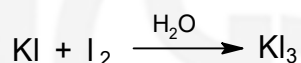
Solution B: Dissolve potassium hydroxide (3 g) in distilled water (40 cm³).

Mix equal volumes of solutions A and B. A white precipitate (Ag₂O) is obtained. Now add concentrated ammonia solution (30%) drop by drop until the mixture is almost clear.

**Schiff's reagent**

Dissolve *p*-rosaniline hydrochloride (0.2 g) in saturated solution (20 cm³) of sulphur dioxide in water. Shake and allow it to stand for few hours until becomes colourless or pale yellow. Dilute with water (180 cm³) and separate the clear solution by filtration.

Iodine-potassium iodide reagent: It is prepared by adding 50 g of potassium iodide and 25 g of iodine to distilled water (200 mL). A clear solution is obtained after stirring. Deep brown colour of the solution is due to the formation of triiodide anion (I₃⁻).

**9.2.4 Procedure**

1. Collect sample(s) of aldehyde and ketone from your counsellor and carry out physical examination of the sample as discussed in BCHCL 134 course.
2. Carry out solubility test as discussed in Sec. 8.3.1. If your compound is soluble in water, check the pH of solution using pH paper.
3. Carry out functional group test (class determination test) as discussed in Sec. 9.2.1.
4. Prepare at least one derivative using procedure discussed in 9.2.2
5. Dry the prepared derivative and take the m.p. of both derivative and sample if it is solid. Predict the actual compound by comparison of melting point of sample and its derivative with literature values. Use appendix A for this purpose.

9.2.5 Observations and Result**1. Physical Examination**

- a) Physical state :
- b) Colour :

Tollen's reagent should be prepared immediately before the experiment because on long standing it decomposes to yield a potentially explosive solid, silver nitride (Ag₃N).

Schiff's reagent tends to acquire colour on storage. Therefore, it is necessary to use only colourless Schiff's reagent.

Keep the Schiff's reagent in a well stopped bottle in dark.

c) Odour :

d) Ignition test :

Comment: This suggests that the unknown 'A' is aromatic/aliphatic compound.

2. **Elemental Analysis:** Not necessary at this point.

3. **Solubility test**

H ₂ O	5% NaOH	5% NaHCO ₃	5% HCl	Conc. H ₂ SO ₄	Expected Class

4. **Test with pH paper:**

5. **Melting point/Boiling point observed:**

5. **Class determination:**

Brady's Test

Tollen's Test

Schiff Test

Iodoform Test

6. **Preparation of derivatives**

a) Derivative chosen

M.P. observed

M.P. reported in literature

b) Derivative chosen

M.P. observed

M.P. reported in literature

7. **Examination of literature**

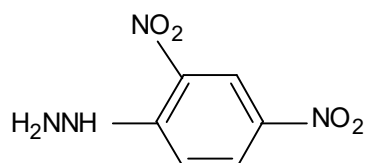
Possible compound.....M.P.....Derivatives

8. The unknown compound is

9. The structure of this compound is

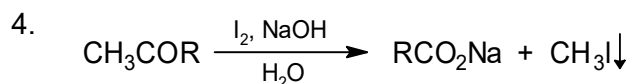
9.3 ANSWERS

1.



2. A German chemist Bernhard Tollens discovered. Tollens test

3. *p*-rosaniline hydrochloride, ii. sulphur dioxide, iii. water



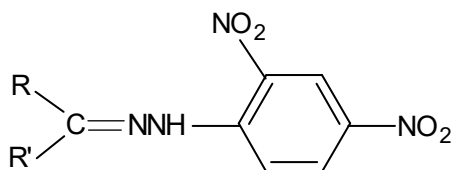
5. a, c and d

6. i) 2,4-Dinitrophenylhydrazone

ii) Semicarbazone

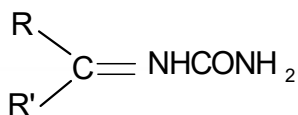
iii) Oximes

7. i)



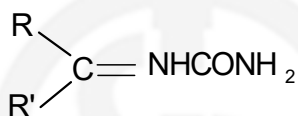
2,4-Dinitrophenyl-hydrazone

ii)



Semicarbazone

iii)



Semicarbazone

EXPERIMENT 10

SYSTEMATIC QUALITATIVE ANALYSIS OF AMIDES, NITRO AND AMINO COMPOUNDS AND PREPARATION OF THEIR DERIVATIVES

Structure

10.1	Introduction		Procedure
	Objectives		Observation and Result
10.2	Experiment 10A: Identification of Amines	10.4	Experiment 10C: Identification of Amides
	Functional Group Tests		Functional Group Tests
	Preparation of Characteristic Derivatives		Preparation of Characteristic Derivatives
	Requirement		Requirement
	Procedure		Procedure
	Observation and Result		Observation and Result
10.3	Experiment 10B: Identification of Nitro Compounds	10.5	Identification of Unknown Samples
	Functional Group Tests	10.6	Laboratory Note Book for Qualitative Analysis of Organic Compounds
	Preparation of Characteristic Derivatives		
	Requirement	10.7	Answers

10.1 INTRODUCTION

Use caution in handling amines. Many are toxic.

In the last experiment we have discussed the qualitative analysis of aldehydes and ketones. In this experiment we shall test the functional group of compounds having i) carbon, hydrogen and nitrogen atoms (amines) and ii) carbon, nitrogen and oxygen atoms (nitro compounds and amides).

First we will learn how to identify amines by i) Hinsberg test ii) Nitrous acid test iii) Diazotisation and iv) Cablyamin test. In the next section we will discussed

the methods of identification of nitro compounds by i) ferrous hydroxide test and ii) by reduction process.

In the last section we will learn how to identify the amide by i) ammonia evaluation test and ii) hydroxamic acid test. We will also learn the method for preparation of derivatives of these compounds.

Expected Learning Outcomes

After performing the experiment given, you should be able to:

- ❖ identify the amines by Hinsberg , nitrous acid, diazotisation and Cablyamin tests;
- ❖ prepare characteristic derivative of amines by Scotten-Baumann method;
- ❖ identify the nitro compounds by ferrous hydroxide test and by reduction process;
- ❖ prepare characteristic derivatives of nitro compounds by reduction to amine;
- ❖ identify the amide by ammonia evaluation test and hydroxamic acid test; and
- ❖ prepare characteristic derivatives of amides by hydrolysis.

10.2 EXPERIMENT- 10a: IDENTIFICATION OF AMINES AND PREPARATION OF THEIR DERIVATIVES

Compounds that contain a basic nitrogen atom with a lone pair are called amines. The substituent -NH_2 is called an amino group. They are formally derivatives of ammonia, wherein one or more hydrogen atoms have been replaced by a substituent such as an alkyl or aryl groups (these may respectively be called alkylamines and arylamines). Amines in which both alkyl and aryl of substituents are attached to one nitrogen atom may be called alkylarylamines.

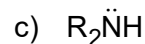
Amines can be classified according to the nature and number of substituents on nitrogen. There are three types of amines i.e. Primary (1°) amines, Secondary (2°) amines and Tertiary (3°) amines

- **Primary (1°) amines:** In primary amines one of three hydrogen atoms in ammonia is replaced by an alkyl or aromatic group.
- **Secondary (2°) amines:** In secondary amines nitrogen atom has one hydrogen atoms and two organic substituents (alkyl, aryl or both) .

- **Tertiary (3°) amines:** In tertiary amines, nitrogen has three organic substituents (alkyl, aryl or both) without any hydrogen atom.

SAQ 1

Classify the following as Primary (1°) amines, Secondary (2°) amines and Tertiary (3°) amines.



10.2.1 Functional Group Test

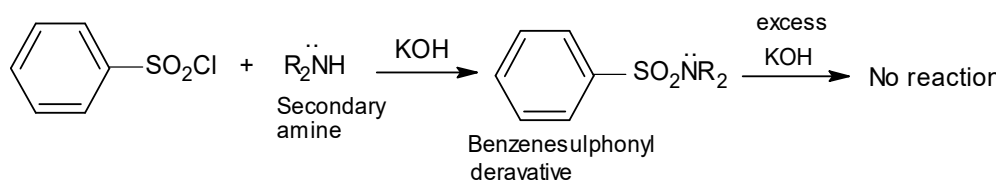
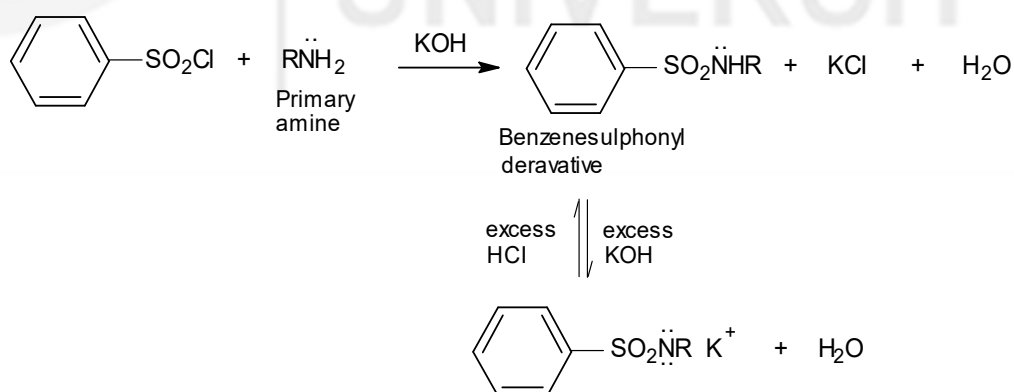
In this section we shall study about the test used for the characterisation of amines. The amines can be mainly identified by (i) Hinsberg Test (ii) Nitrous Acid Test (iii) Diazotisation and Coupling and (iv) Cabylamine test (Positive for primary amines).

i) Hinsberg Test

Primary, secondary and tertiary amines can be identified by Hinsberg test. These three amines can be distinguished by this test. The reaction between primary or secondary amines and benzenesulfonyl chloride gives the corresponding substituted benzenesulfonamide. The reaction is carried out in excess base. Sulfonamides, of primary and secondary amines, are distinguishable because sulphonamides from primary amine have acidic amino hydrogen, which make the product soluble in alkali. On the other hand, the benenesulfonamide secondary amines bear no acidic aminmo hydrogen and they are insoluble in both acid and base. Tertiary amines lack the necessary acidic hydrogen for formation of benzenesulfonyl derivatives.

Do you allow benzenesulfonyl chloride to come in contact with skin. Use caution in handling amines. Many of these are toxic.

Thus, with few exceptions, primary amines react with benzenesulfonyl chloride to provide homogeneous reaction mixtures and secondary amines react to yield heterogeneous reaction mixtures.



Procedure

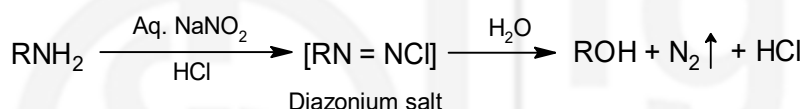
In a test tube take 3-4 drops (or 0.1 g) of the unknown compound, 0.2 g of benzenesulfonyl chloride 1 cm³ of methanol and 5 cm³ of 10% sodium hydroxide. Heat the mixture for few minutes. Just below its boiling point, till order of benzenesulfonyl chloride is gone. Then cool the reaction mixture in ice. On cooling, if no precipitate appears than the substance is probably a tertiary amine. If precipitate appears, the amine is either primary or secondary.

If a precipitate is present, filter it, wash it with 2 cm³ of water and transfer it to a test tube. Add 2 cm³ of 5% sodium hydroxide solution and warm the reaction mixture to 50° C and shake it well. If the precipitate dissolves, the amine is primary. If the precipitate does not dissolve it indicates a secondary amine.

ii) Nitrous Acid Test

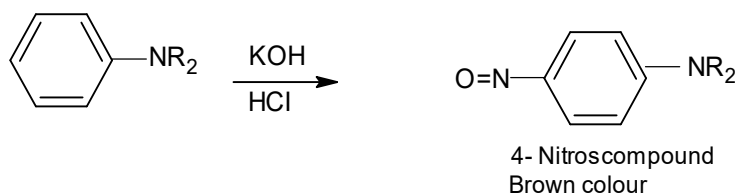
The nitrous test is useful to differentiate between primary aliphatic, primary aromatic, secondary and tertiary amines.

Primary amines react with nitrous acid to yield diazonium ion. The aliphatic amines yield unstable diazonium ion which decomposes to give nitrogen gas and alcohol. On the other hand aromatic amines give stable diazonium salt (stable in solution at 0° C).

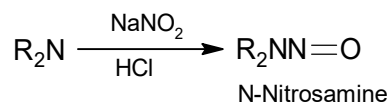
**Procedure**

Take 0.5 g of the compound in a test tube and dissolve it in 2 cm³ 2 M hydrochloric acid (Note: few weakly basic amines require conc. Hydrochloric acid). Cool it to 5°C in ice and add 4 or 5 drops of 5% aqueous sodium nitrite. Note which of the following is observed:

- i) There is effervescence (nitrogen gas is evolved) and clear solution is obtained. This shows the presence of primary aliphatic amine or amide (RCONH₂).
- ii) No effervescence and formation of a clear solution indicates the presence of primary aromatic amine or tertiary amine.
- iii) Formation of dark brown solution indicates the presence of tertiary aromatic amine.



- iv) No effervescence but the formation of cloudy solution or emulsion (generally yellow) indicates the presence of secondary amine.



iii) Diazotisation and Coupling

The **diazo** group is an organic moiety consisting of two nitrogen atoms (azo) at the terminal position. The general structural formula is $\text{R}_2\text{C}=\text{N}^+=\text{N}^-$. They are called diazo compounds or diazoalkanes. The process of conversion of primary aromatic amines into its diazonium salt is called diazotization or diazotation. Diazonium salts are important synthetic intermediates that can undergo coupling reactions to form azo dyes. Reaction between primary aromatic amine and nitrous acid give a diazo compound. This reaction is known as diazotization. It was first given by Peter Griess. Diazonium salt is used at very low temperature because of the fact that at the higher temperature they are unstable. Secondary and tertiary amines do give this test. Secondary amine has only have one hydrogen atom on the nitrogen and hence they cannot complete the diazotization reaction and gives yellow oily nitrosamine products. Tertiary amines do not have any hydrogen atom on nitrogen; simply undergo an acid–base reaction with nitrous acid giving soluble salts. Many diazonium salts are highly explosive and decompose violently on heating. One must be very careful while using them.

Destroy the isocyanide by adding excess of hydrochloric acid and throw it outside.

Anilides ($\text{C}_6\text{H}_5\text{NHCOR}$) also give positive carbylamines test.

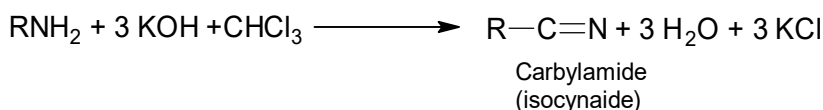
Procedure

If nitrous acid test gives a clear solution, then to this solution add 5% 2-naphthol dissolved in sodium hydroxide (2M) and note which of the following is observed:

- Formation of bright red to dark brown precipitate indicates the presence of primary aromatic amine.
- If no colour appears (ignore white to yellow precipitate) and test (ii) (a) is positive then perform carbylamines test for primary amines.

iv) Carbylamine test: Positive for primary amines

The carbylamine reaction is used for the identification of primary amines. This is also known as Saytzeff's isocyanide test. In this reaction, the analyte is heated with alcoholic potassium hydroxide and chloroform. Formation of isocyanide (carbylamine) indicates the presence of a primary amine, the presence of an amine being indicated by the characteristic foul smell of an isocyanide. Secondary and tertiary amines do not give carbylamine test, since they have more than one alkyl group which hinders the approach of $-\text{NH}_2$ group. The carbylamines reaction, also known as Hoffman's isocyanide test



Procedure

Place a small quantity of organic compound, alcoholic KOH (caustic potash) solution (1 cm^3) and chloroform (few drops) in a test tube. Shake the contents

of tube and heat gently. A bad smell of isocyanide indicates the presence of a primary amine.

SAQ 2

Fill in the following blanks.

- The reaction between primary and benzenesulfonyl chloride gives
- Nitrous Acid test is useful to differentiate between, ... and amines.
- Primary amine on reaction with alcoholic caustic potash in presence of chloroform gives
- Cabylamine test gives positive for amines

10.2.2 Characteristic Derivatives

The most common derivatives of primary and secondary amines are benzoate and toluene -4- sulphonate. Acetyl derivative is also quite common. However, tertiary amines do not undergo same reactions. Solid derivatives suitable for characterisation of tertiary amines are the picrate and methiodes.

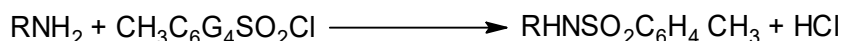
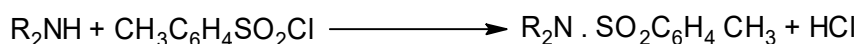
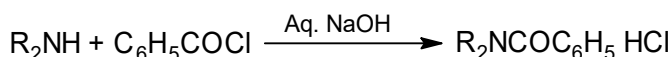
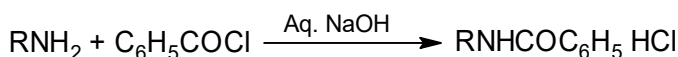
Experimental procedures for the preparation of these derivatives are given below:

Procedure

i) Benzoate and Toluene-4 sulphonate (Scotten-Baumann Method)

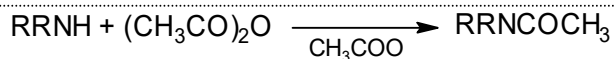
Place the compound (0.5 g), 2 M sodium hydroxide (10 cm³) and benzoylchloride (1 cm³) in a boiling tube. If the mixture is not homogenous, add sufficient amount acetone. Shake the contents vigorously until a solid is obtained. Sometimes few drops of water is added in order to get the precipitate. Filter off the precipitate, wash with cold water and recrystallise from alcohol.

If you want to prepare toluene -4- sulphonate, use toluene -4- sulphonyl in place of benzoylchloride.



ii) Acetyl Derivative

Primary and secondary amines are best acetylated with acetic anhydride.



R = alkyl, aryl or H

Procedure

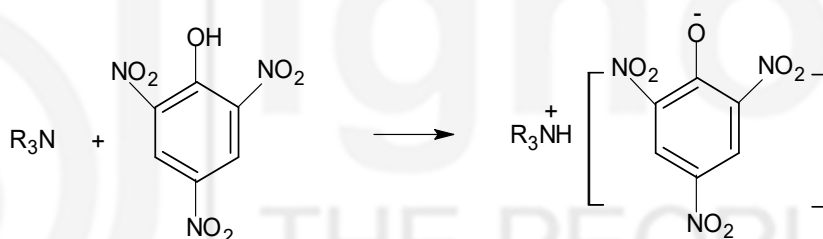
Place amine (0.5 g), acetic acid (1 cm³) and acetic anhydride (1 cm³) in a conical flask. Heat the reaction mixture for about 20 minutes, cool and pour the contents into ice-water. Filter the solid and crystallise from water or aqueous ethanol.

Some *ortho* substituted derivatives of aromatic amines are difficult to derivatise because of steric hindrance. Such derivatives can be prepared by adding a few drops of concentrated sulphuric acid, which acts as a catalyst, and by use of an excess of acetic anhydride.

iii) Picrates

Procedure

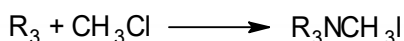
Dissolve the amine (0.5 g) in ethanol (10 cm³) and add saturated ethanolic solution of picric acid (5 cm³). Heat the reaction mixture on water bath for 3 minutes and allow it to cool. Filter the solid product and recrystallize from ethanol.



iv) Methiodides

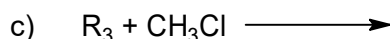
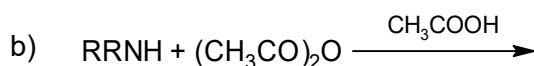
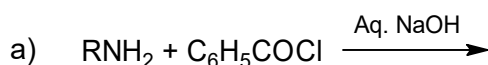
Procedure

Gently Warm a mixture of the amine (0.5 g) with methyl iodide (0.5 g cm³) on a water bath for several minutes. Then cool it in an ice and recrystallise the product from ethanol or methanol or ethyl acetate.



SAQ3

Complete the following reactions;



SAQ 4

Tick the correct answer from the following choices given below:

a) Carbylamine test is responded by:

- i) $\text{CH}_3\text{CH}_2\text{NH}_2$
- ii) $\text{CH}_3\text{NHC}_2\text{H}_5$
- iii) $(\text{CH}_3)_3\text{N}$
- iv) $(\text{C}_6\text{H}_5)_2\text{NH}$

b) Nitrosoamines are formed by

- i) Primary amine
- ii) secondary amine
- iii) tertiary amine
- iv) none of above

10.2.3 Requirements

S. No.	Apparatus	Qty.	S. No.	Chemicals
1	Beakers	1	1	HCl
2	Boiling tube	1	2	KOH
3	Burner	1	3	NaOH
4	Conical flask	1	4	Acetic acid
5	Droppers	1	5	Acetic anhydride
6	M.P. Apparatus	1	6	Aqueous sodium nitrite
7	Reflux condenser	1	7	Benzenesulfonyl chloride
8	Round bottom flask	1	8	Benzoylchloride
9	Test tubes	3	9	Chloroform
10	Thermometers	1	10	Ethanol
11	Water bath	1	11	Ethyl acetate
			12	ice
			13	Methanol
			14	Methyliodide
			15	2-Napthol
			16	Picric acid
			17	Toluene -4- sulphonyl
			18	Samples of amines

10.2.4 Procedure

1. Collect sample(s) of amine from your counsellor and carry out physical examination and also elemental test of the sample as discussed in BCHCL 134 course.
2. Carry out solubility test as discussed in Sec. 8.3.1. If your compound is soluble in water, check the pH of solution using pH paper.
3. Carry out functional group test (class determination test) as discussed in Sec. 10.2.1.
4. Prepare at least one derivative using procedure discussed in 10.2.2
5. Dry the prepared derivative and take the m.p. of both derivative and sample if it is solid. Predict the actual compound by comparison of melting point of sample and its derivative with literature values. Use appendix A for this purpose.

10.2.5 Observations and Result

1. Physical Examination

- a) Physical state :
- b) Colour :
- c) Odour :
- d) Ignition test :

Comment: This suggests that the unknown 'A' is aromatic/aliphatic compound.

2. Elemental Analysis: Nitrogen test:

3. Solubility test

H ₂ O	5% NaOH	5% NaHCO ₃	5% HCl	Conc. H ₂ SO ₄	Expected Class

4. Test with pH paper:

5. Melting point/Boiling point observed:

6. Class determination:

- (i) Hinsberg Test
- (ii) Nitrous Acid Test
- (iii) Diazotisation and Coupling and
- (iv) Cabylamine test

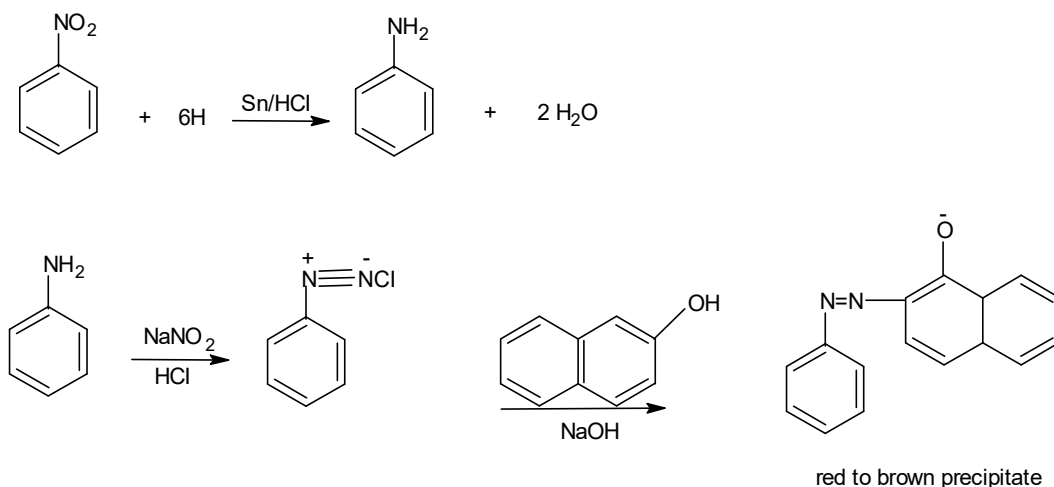
7. Preparation of derivatives

- a) Derivative chosen
M.P. observed
M.P. reported in literature

10. The structure of this compound is

ii) Reduction to Amines and Dye Test

Nitro compounds can identify by reduction of nitro group. The reaction involved is:



Procedure

Take about 0.5 g of the unknown compound and 3 cm^3 of 7 M hydrochloric acid in a boiling tube. Add 1 g of stannous chloride to it and warm with continuous shaking for 15 minutes. Filter the mixture, cool it in ice (5°C) and add 5% aqueous sodium nitrite (5-6 drops). To this add 2 cm^3 of 5% solution of 2-naphthol in 2 M sodium hydroxide and note which of the following happens:

- A red to brown precipitate forms which indicates the presence of aromatic nitro compounds.
- No coloured precipitate formation which indicates the presence of aliphatic nitro compounds. (Ignore white to yellow precipitate).

10.3.2 Characteristic Derivatives

Preparation of derivatives of nitro compounds depends on the type of nitro compounds detected. Nitro compounds are reduced to amines and derivatized as primary amines. This method can be utilized for both aliphatic and aromatic nitro compounds.

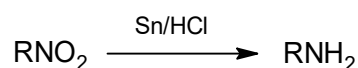
Reduction of Nitro compounds to Primary Amines

Preparation of derivatives of nitro compounds depends on the type of nitro compounds. Nitro compounds are reduced to amines and derivatised as primary amines. This method can be utilised for both aliphatic and aromatic nitro compounds. Nitro compounds can be converted to primary amines by the following method.

Procedure

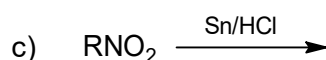
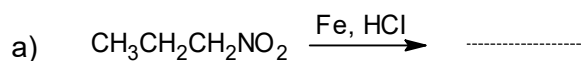
Place the nitro compound (1 g) and concentrated hydrochloric acid (10 cm^3) into a small round bottom flask and add ethanol (2 cm^3) and tin (3 g). Cool the reaction mixture until initial reaction subsides and then heat under reflux for 25 minutes. Decant the supernatant liquid, cool it and basify with 40% sodium hydroxide with stirring and cooling in ice (use excess of 40% Sodium hydroxide to dissolve tin (II) hydroxide formed). Extract the alkaline

mixture with diethyl ether and, dry on anhydrous sodium sulphate, filter and then evaporate ether. Crystallize the solid from aqueous ethanol.



SAQ 5

Fill up the blanks:



10.3.2 Requirements

S. No.	Apparatus	Qty.	S. No.	Chemicals
1.	Test tubes	3	1.	NaOH
2.	Droppers	1	1.	H ₂ SO ₄
3.	Water bath	1	2.	HCl
4.	Thermometers	1	3.	Sodium nitrite
5.	Beakers	2	4.	Ethanol
6.	M.P. Apparatus	1	5.	Diethyl ether
7.	Round bottom flask	1	6.	Phenolphthalein
8.	Reflux condenser	1	7.	Ferrous ammonium sulphate
9.	Conical flask		8.	Sodium potassium hydroxide
10.	Burner	1	9.	Stannous chloride
11.	Boiling tube	1	10.	Tin
12.	Erlenmeyer flask.		11.	Samples of nitro compound

10.3.3 Procedure

1. Collect sample(s) of nitro compound from your counsellor and carry out physical examination and also elemental test of the sample as discussed in BCHCL 134 course.

2. Carry out solubility test as discussed in Sec. 8.3.1. If your compound is soluble in water, check the pH of solution using pH paper.
3. Carry out functional group test (class determination test) as discussed in Sec. 10.3.1.
4. Prepare at least one derivative using procedure discussed in 10.3.2
5. Dry the prepared derivative and take the m.p. of both derivative and sample if it is solid. Predict the actual compound by comparison of melting point of sample and its derivative with literature values. Use appendix A for this purpose.

10.3.5 Observations and Result

1. Physical Examination

- a) Physical state :
- b) Colour :
- c) Odour :
- d) Ignition test :

Comment: This suggests that the unknown 'A' is aromatic/aliphatic compound.

2. Elemental Analysis: Nitrogen test:

3. Solubility test

H ₂ O	5% NaOH	5% NaHCO ₃	5% HCl	Conc. H ₂ SO ₄	Expected Class

4. Test with pH paper:

5. Melting point/Boiling point observed:

6. Class determination:

- (i) Ferrous hydroxide test and
- (ii) Reduction to amines and Dye Test.

6. Preparation of derivatives

- a) Derivative chosen
M.P. observed
M.P. reported in literature
- b) Derivative chosen
M.P. observed
M.P. reported in literature

7. Examination of literature

Possible compound.....M.P.....Derivatives

8. The unknown compound is

9. The structure of this compound is

10.4 EXPERIMENT 10c: IDENTIFICATION OF AMIDES AND PREPARATION OF THEIR DERIVATIVES

Amides are represented by the general formula RCONH_2 . The qualitative test generally used for amides are ammonia evolution test and hydroxamic acid test.

10.4.1 Functional Group Test

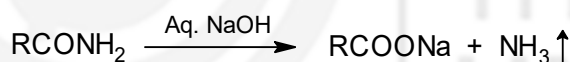
There are two main functional group tests for identification of amides.

- i) Ammonia Evolution Test and
- ii) Hydroxamic Acid Test.

i) Ammonia Evolution Test

Amides are hydrolysed with aqueous sodium hydroxide to carboxylic acid salt and ammonia. The evolution of ammonia is indicative of amide.

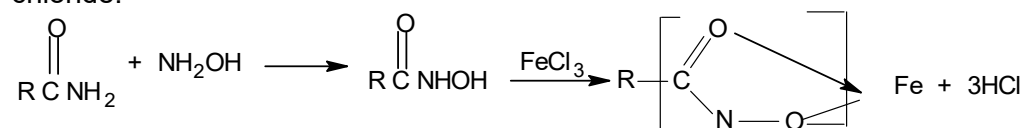
This test fails if hydrogen at the nitrogen atom is replaced by an alkyl or aryl group, then amines are produced.

**Procedure**

Heat about 0.2 g of the compound with 2 cm³ of 2 M aqueous sodium hydroxide. Evolution of ammonia indicates the presence of an amide group. Detect the evolution of ammonia by its smell and/or by its action on moist red litmus paper (red → blue).

ii) Hydroxamic Acid Test

Amides, give a coloured solution on treatment with hydroxylamine and ferric chloride.

**Procedure**

Take 2-3 drop or 0.02 g of sample compound in a boiling tube and add 0.2 g of solid hydroxylamine hydrochloride and 5 cm³ 10% NaOH solution. Heat the mixture on a boiling water bath. Cool and acidify the reaction mixture with

dilute hydrochloric acid and add 2-3 drops of 5% aqueous ferric chloride. Purple or deep red colour due to formation of ferric complex of hydroxamic acid indicates the presence of amide.

10.4.2 Characteristic Derivatives

Primary amide (RCONH_2) on hydrolysis furnishes carboxylic acid. Carboxylic acid is identified to characterize the amide.



i) Hydrolysis of Amide-Isolation of Acid

Place the unknown compound (0.5 g) and 2 M aqueous sodium hydroxide (10 cm^3) into a conical flask and heat the reaction mixture on water bath for about 30 minutes. The reaction mixture is cooled and acidified with dilute sulphuric acid. The precipitated acid is filtered, washed with water and recrystallize from water or ethanol. If on acidification no precipitate is formed, use it for the preparation of acid derivatives.

ii) Picrate Derivatives

Some amides form picrates. Prepare picrate derivatives as described under 10.3.2 (iii).

SAQ 6

Fill in the blanks:

- Benzamide upon hydrolysis with alkali followed by acidification furnished and
- Ethanoic acid on reaction with ammonia afforded

10.4.3 Requirements

S. No.	Apparatus	Qty.	S. No.	Chemicals
1.	Test tubes	3	1.	Ethanol
2.	Droppers	1	2.	Sulphuric acid
3.	Water bath	1	3.	Sodium hydroxide
4.	Thermometers	1	4.	Potassium hydroxide
5.	Beakers	2	5.	Ferric chloride
6.	M.P. Apparatus	1	6.	Hydroxylamine hydrochloride
7.	Round bottom flask	1	7.	Hydrochloric acid
8.	Reflux condenser	1	8.	

9.	Conical flask		9.
10.	Burner	1	10.
11.	Boiling tube	1	11.
12.	Erlenmeyer flask.		12.

10.4.4 Procedure

1. Collect sample(s) of nitro compound from your counsellor and carry out physical examination and also elemental test of the sample as discussed in BCHCL 134 course.
2. Carry out solubility test as discussed in Sec. 8.3.1. If your compound is soluble in water, check the pH of solution using pH paper.
3. Carry out functional group test (class determination test) as discussed in Sec. 10.3.1.
4. Prepare at least one derivative using procedure discussed in 10.3.2
5. Dry the prepared derivative and take the m.p. of both derivative and sample if it is solid. Predict the actual compound by comparison of melting point of sample and its derivative with literature values. Use appendix A for this purpose.

10.3.5 Observations and Result

1. Physical Examination

- a) Physical state :
- b) Colour :
- c) Odour :
- d) Ignition test :

Comment: This suggests that the unknown 'A' is aromatic/aliphatic compound.

2. Elemental Analysis: Nitrogen test:

3. Solubility test

H ₂ O	5% NaOH	5% NaHCO ₃	5% HCl	Conc. H ₂ SO ₄	Expected Class

4. Test with pH paper:

5. Melting point/Boiling point observed:

6. Class determination:

- i) Ammonia Evolution Test
- ii) Hydroxamic Acid Test

7. Preparation of derivatives

- a) Derivative chosen
M.P. observed
M.P. reported in literature
- b) Derivative chosen
M.P. observed
M.P. reported in literature

8. Examination of literature

Possible compound.....M.P.....Derivatives

- 9. The unknown compound is
- 10. The structure of this compound is

10.5 IDENTIFICATION OF UNKNOWN SAMPLES

After having good practice with the known functional group compound collect few samples for the unknown compounds from the category of functional group discussed in this course. Follow the steps given below:

- 1. Physical examination
 - a) Physical State
 - b) Colour
 - c) Odour
 - d) Ignition test
- 2. Physical constants
Melting point/Boiling point
- 3. Element detection
(N,S, halogen)
- 4. Solubility test
- 5. Functional Group test
- 6. Preparation of derivatives
Prepare suitable derivatives of the proposed compound and compare the melting point with the literature report.
- 7. Examination of literature
Compare the information obtained so far (1-6) with the literature report.
- 8. Write correct name and structure of the compound

Various experimental steps may be recorded in systematic way for the identification of compound. This may be illustrated by taking the example of 2-Naphthol.

10.6 LABORATORY NOTE BOOK FOR QUALITATIVE ANALYSIS OF ORGANIC COMPOUNDS

Here we will discuss some important points one should keep in mind while preparing a laboratory note book for qualitative analysis of organic compounds.

Each experiment should start on a fresh page, which should contain a title and experiment number at the top. A sample note book format for qualitative experiment is given here. Identification of 2-naphthol (β -naphthol) is taken as our example.

Experiment No. 'A' Identification of an Unknown Organic Compound

1. Physical examination

- a) Physical state.....Solid
- b) Colour.....white
- c) Odour.....Faint phenol-like odor
- d) Ignition test luminous, sooty flame, no residue

Comments: This suggest that the compound 'A' aromatic compound.

2. Physical Constants

Melting point observed 122-124°C

3. Elemental Analysis

N,S,Cl,Br, I.....None

4. i) Solubility test

H ₂ O	Aq. NaOH	Aq.NaHCO ₃	HCl	Conc.H ₂ SO ₄	Expected class
	Soluble phenols	Slightly soluble	-	-	Aromatic
	No bubbles				

5. Reaction to pH paper not visible colour change

6. Functional Group Test

- i) With alcoholic FeCl₃.....Green solution.

Comment: The above tests indicated the presence of phenolic compound

7. Preparation of Derivatives

a) Derivatives chosen 3,5-Benzoate

M.P. Observed 208-210°C

M.P. reported 210°C

b) Derivative chosen 1-Naphthylurethane

M.P. observed 156-158°C

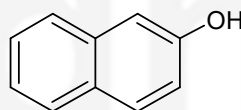
M.P. in literature 157°C

8. Examination of literature

M.P. of Derivatives			
Possible compound	M.P	3,5-benzoate	1-Naphthylurethane
2-Naphthol		M.P.	M.P.
	123°C	210°C	157°C

Comment: The above data correspond to 2-naphthol

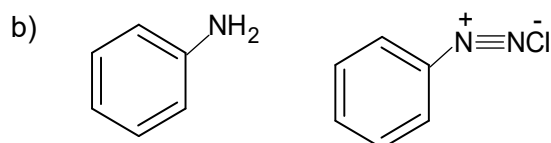
9. Result: The unknown compound is 2-naphthol. The structure of this compound is

**10.7 ANSWERS****Self Assessment Questions**

1. a) primary amine, b) tertiary amine, c) secondary amine

2. a) benzenesulfonamide, b) primary, secondary, tertiary
c) isocyanide d) primary3. a) $\text{RNHCOC}_6\text{H}_5 + \text{HCl}$ b) RRNCOCH_3 c) $\text{R}_3\text{NCH}_3\text{I}$

4. a) i b) ii

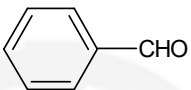
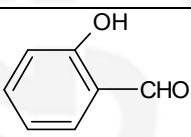
5. a) $\text{CH}_3\text{CH}_2\text{CH}_2\text{NH}_2$ c) RNH_2

6. a) benzoic acid; ammonia

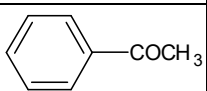
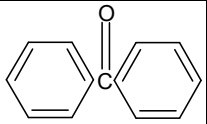
b) ethanamide

APPENDIX Tables for B.P./M.P. of Organic Compounds and their Derivatives

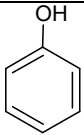
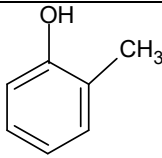
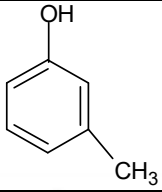
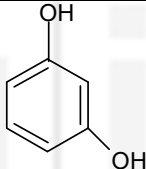
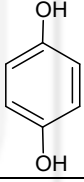
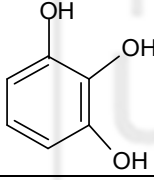
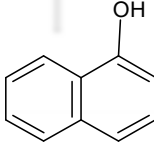
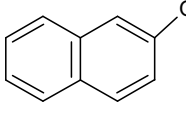
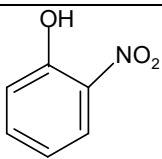
Aldehyde and their derivatives

Compound	Formula	B.P./ M.P. * (°C)	<u>B.P./ M.P. of Derivatives</u> (°C)		
			2,4-Dinitrophenyl hydrazone	Semica bazone	Oxime
Formaldehyle	HCHO	-21	166	169 with decomposition	
Acetaldehyde	CH ₃ CHO	20	168	169	47
Propionaldehyd e	CH ₃ CH ₂ CHO	49	150	89	40
Benzaldehyle		179*	236	222	35
Salicyladehyde		196*	252 Decomposed	231	63

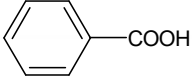
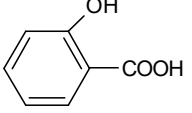
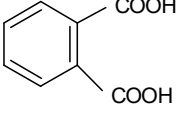
Ketones and their derivatives

Compound	Formula	B.P./ M.P. * (°C)	<u>M.P. of Derivatives (°C)</u>		
			2,4-Dinitrophenyl hydrazone	Semicar bazone	Oxime
Acetone	CH ₃ COCH ₃	56	128	190	59
Ethylmethyl ketone	CH ₃ COC ₂ H ₅	80	115	145	
Acetophenone		202	240	199	60
Benzophenone		48*	239	165	143

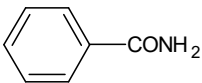
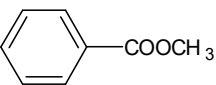
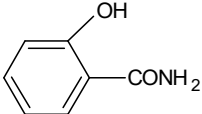
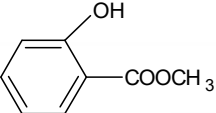
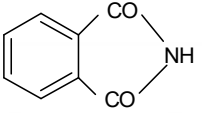
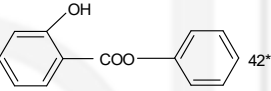
Phenols and their derivatives

Compound	Formula	B.P./ M.P.* (°C)	M.P. of Derivatives (°C)	
			2,4-Dinitrophenyl hydrazone	Semicabazone
Phenol		182	146	133
<i>o</i> -Cresol		191	138	142
<i>m</i> -Cresol		202	165	128
<i>p</i> -Cresol		201	189	146
Resorcinol		118*	201 [Disubstituted] 206	
Hydroquinon or Quinol		171*	317	247
Pyrogallol		132*	205	
1-Naphthol		95*	217	152
2-Naphthol		123*	210	157
<i>o</i> -Nitrophenol		45*	155	113

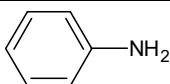
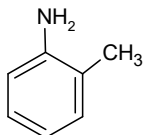
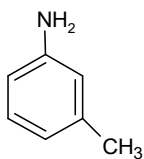
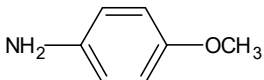
Carboxylic Acids and their Derivatives

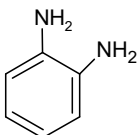
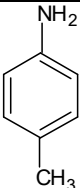
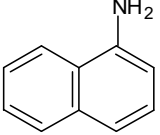
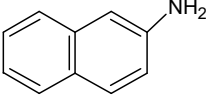
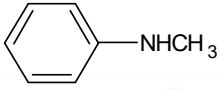
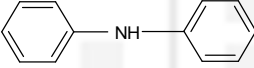
Compound	Formula	B.P./ M.P.* (°C)	<u>M.P. of Derivatives (°C)</u>		
			Amide	Anilide	<i>p</i> -toluidide
Formic acid	HCOOH	101	---	50	53
Acetic acid	CH ₃ COOH	118	82	114	147
Propenoic acid	CH ₃ CH ₂ COOH	140	85	105	141
Undecanoic acid	CH ₃ (CH ₂) ₉ COOH	26*	123	99	125
Oxalic acid (Dihydrate)	$\begin{array}{c} \text{COOH} \\ \\ \text{COOH} \end{array} \cdot 2\text{H}_2\text{O}$	101*	419d	254	268
Citric acid (Hydrated)	$\begin{array}{c} \text{CH}_2\text{COOH} \\ \\ \text{C}(\text{OH})\text{COOH} \\ \\ \text{CH}_2\text{COOH} \end{array}$	100*	210	192	189
Tartaric acid	$\begin{array}{c} \\ \text{CH}(\text{OH})\text{COOH} \\ \\ \text{CH}(\text{OH})\text{COOH} \end{array}$	169*	196d	246d	--
Succinic acid	$\begin{array}{c} \text{CH}_2\text{COOH} \\ \\ \text{CH}_2\text{COOH} \end{array}$	189*	260	230	255
Benzoic acid		121*	130	160	158
Salicylic acid		158*	139	135	
Phthalic acid		210*	200	253	201

Ester and Amides

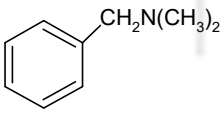
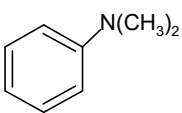
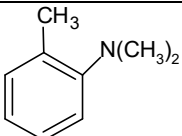
Compound	Formula	B.P./ M.P.* (°C)	Amide	Formula	M.P.
Methyl acetate	CH_3COOH_3	57	Acetamide Urea	CH_3CONH_2 NH_2CONH_2	82
Ethyl acetate	$\text{CH}_3\text{COOC}_2\text{H}_5$	77	N-Methylurea	$\text{CH}_3\text{NHCONH}_2$	132
Diethyl Oxalate	COOC_2H_5 COOC_2H_5		N,N-Dimethyl urea	$\text{CH}_3\text{NHCONHCH}_3$	102
Methyl Oxalate	COOCH_3 COOCH_3	186	Benzamide		182
Methyl benzoate		54*	Salicylamide		129
Methyl salicylate		199	Phthalimide		139
Phenyl salicylate		224			235

Primary and Secondary Amines and their derivatives

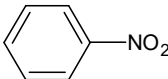
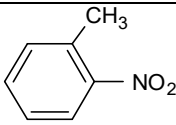
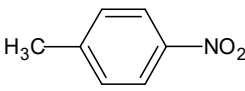
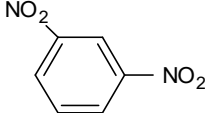
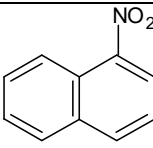
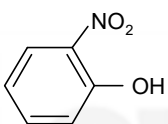
Compound	Formula	B.P./ M.P.* (°C)	M.P. of Derivatives (°C)			
			Benzoate	Picrate	Toluen e-4- sulpho nate	Acet- amide
Primary Amines						
1-Aminopropane	CH ₃ CH ₂ CH ₂ NH ₂	49	84	135	52	
Aniline		181	163	...	103	114
o-Toluidine		200	144	213	110	112
m-Toluidine		203	125	200	114	66
o-Anisidine		225	60	200	127	88

o-Phenetidine		229	104	...	164	79
p-Toluidine		45*	158	181	118	154
1-Aminonaphthalene		50*	161	163	157	160
2-Aminonaphthalene		113*	162	195	133	134
Secondary Amines						
Diethylamine	(C ₂ H ₅)NH	56	42	155	60	...
N-Methylaniline		193	63	145	95	
Diphenylamine		54*	180	182	142	

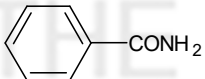
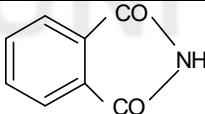
Tertiary Amines and their Derivatives

Compound	Formula	B.P./ (°C)	B.P./ M.P. of Derivatives (°C)	
			Picrate	Methiodides
Trimethylamine	(CH ₃) ₃ N	33	216	230
Triethylamine	(C ₂ H ₅) ₃ N	89	173	...
N,N-Dimethylbenzylamine		184	93	179
N,N-Dimethylaniline		193	164	228
N,N-Diemthyl-o-toluidine		185	122	210

Nitro compounds

Compound	Formula	B.P./M.P. (°C)
1-Nitropropane	$\text{CH}_3\text{CH}_2\text{CH}_2\text{NO}_2$	131
Nitrobenzene		211
o-Nitrotoluene		222
p-Nitrotoluene		54*
m-Dinitrobenzene		90*
Nitronaphthalene		61*
o-Nitrophenol		45*

Amides

Compound	Formula	B.P./M.P. (°C)
Acetamide	$\text{CH}_3\text{CH}_2\text{CH}_2\text{NO}_2$	82
Palmitamide	$\text{CH}_3(\text{CH}_2)_{14}\text{CONH}_2$	106
Benzamide		129
M-Methylurea	$\text{CH}_3\text{NHCONH}_2$	102
Urea	NH_2CONH_2	132
Phthalimide		238

EXPERIMENT 11

DIFFERENTIATION BETWEEN REDUCING SUGARS AND NON-REDUCING SUGARS

Structure

11.1	Introduction	11.4	Procedure
	Expected Learning Outcomes	11.5	Observation
11.2	Principle	11.6	Result
11.3	Requirement		

11.1 INTRODUCTION

You have already studied the chemistry theory of carbohydrate in the BCHCT-135 Course. Carbohydrate, you should know by now, are compound of carbon, hydrogen and oxygen having empirical formula such as $C_n(H_2O)_n$ or $C_n(H_2O)_{n-1}$. These formulas suggest that they are hydrates of carbon and that is why early chemist gave the general name carbohydrates. We commonly call carbohydrates sugars and they also known as saccharides. Having studied the chemistry of carbohydrates in your theory course, now in the experiment we will perform some tests to distribution reducing and non conducting sugars.

Expected Learning Outcomes

After performing the experiment given, you should be able to:

- ❖ define monosaccharides, disaccharides and polysaccharides;
- ❖ explain the chemistry of various qualitative tests used to distinguish reducing and non reducing sugar;
- ❖ perform different tests such as Fehling's Test, Benedict's Test and Barfoed's Test to distinguish reducing and non-reducing sugars.

11.2 PRINCIPLE

Before going in further details of this experiment let us recapitulate some main points related to the chemistry of carbohydrates. Carbohydrates are actually

polyhydroxy aldehydes and polyhydroxy ketones, therefore they are also referred as aldoses and ketoses, respectively. As you have studied in your theory course, carbohydrates are divided into three general classes, depending on the number of carbohydrate molecular units they contain: Monosaccharides are carbohydrates that cannot be broken into smaller units upon hydrolysis. They may contain between 3 and 7 carbons. Common examples of monosaccharides are glucose and fructose. Oligosaccharides upon hydrolysis yield between 2-6 monosaccharide units and, depending upon the number of monosaccharide units so obtained, are known as disaccharides, trisaccharides, etc. Common examples of this class are sucrose and lactose. Polysaccharides produce a large number of monosaccharide units (more than six) upon hydrolysis. They may contain as many as 3000 monosaccharide units. Starch and cellulose are common examples of polysaccharides

Six carbon monosaccharides, such as glucose undergoes intramolecular reaction between a hydroxyl group and the carbonyl group to form hemiacetal form. We refer to the carbonyl carbon involved in the reaction as the **anomeric carbon**. At this carbon atom there are two stereo chemical configuration possible, alpha- (α) and beta- (β). The two molecules containing these configurations are referred to as **anomers**. The α - and β -forms may be interconverted by a process called mutarotation, which proceeds via the acyclic aldehyde (see Fig. 11.1). In aqueous solution, an equilibrium mixture contains 36% α -anomer and 64% β -anomer. The two forms undergo interconversion through the open form of glucose (0.02%). A similar intramolecular reaction occurs between a hydroxyl group and the ketone group of fructose to form a hemiketal.

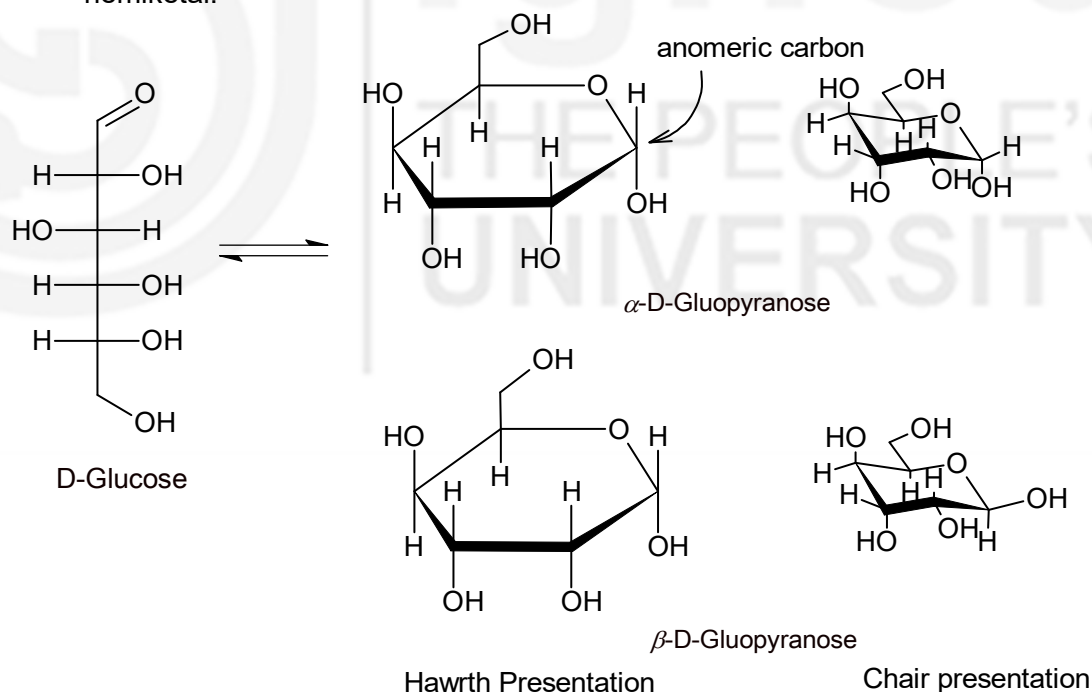


Fig. 11.1: Anomers of Glucose.

Carbohydrate can qualitatively be characterized on the basis of their reactions with dehydrating acid (H_2SO_4) and condensation reagents such as phenols. Molisch's test, Seliwanoff test and Bial test come in this category. These tests are used to distinguish between carbohydrates and non-carbohydrates; aldose and ketose sugars; and pentose and hexose sugars. Second class of

reagents are oxidizing agents such as solution of Cu(II) ions. In this experiment we will focus on the oxidation reactions of carbohydrates with Copper(II) ions solutions. Three common reagents of this class are Fehling's solution, Benedict's solution and Barfoed's reagent. These reagents are mild oxidizing agents and consist of solutions contain Copper(II) ions which oxidize contain carbohydrates.

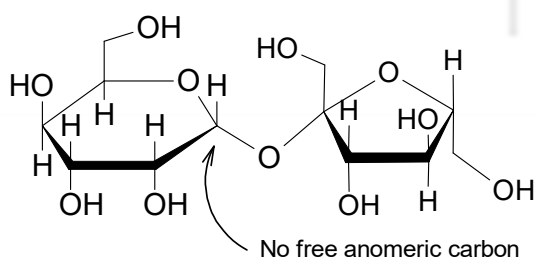
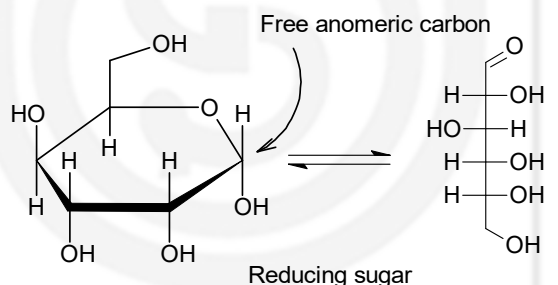
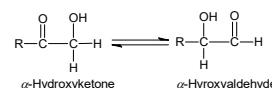
Alkaline Cu(II) ions solution readily oxidize many mono- and disaccharides with the oxidation of the sugar molecules. In this process, there is a simultaneous reduction of the Copper(II) ions $[\text{Cu}^{2+}]$ to a copper(I) ions $[\text{Cu}^+]$. This change in reaction can be identified easily by the colour change from a deep blue to a red or reddish brown.



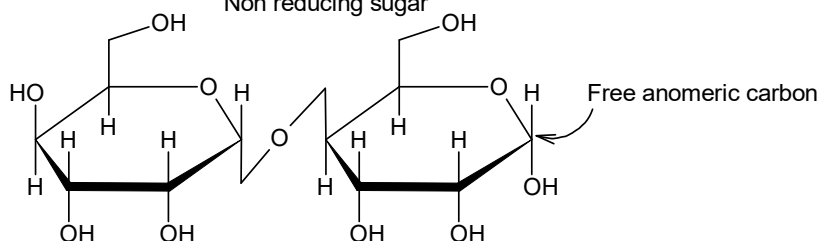
Reducing and Non-reducing Sugar

Any Sugar which is capable of being oxidized and causes the reduction of oxidizing agents such as a Copper(II) ions (Cu^{2+}) to copper(I) ions (Cu^+) is known as reducing sugar, but those which are unable to be oxidized and do not reduce oxidizing agents are known as non-reducing sugars. All monosaccharides are reducing sugar, they all have a free reactive carbonyl group in the terms of aldehyde or cyclic hemiacetal. We can also distinguish between reducing sugar and non-reducing sugars on the basis of presence of anomeric carbon. If the oxygen on the anomeric carbon of a sugar is not attached to any other structure, that sugar can easily change to aldose and can act as a reducing agent, which is termed a reducing sugar.

Both aldose and ketose sugars can be reducing sugars. Under reaction conditions, ketose sugars undergo base-catalyzed rearrangement reaction to aldose sugars, which subsequently react with oxidizing agent such as Fehling's, Benedict's or Barfoed's reagents.



Sucrose
Non reducing sugar



Lactose
Reducing sugar

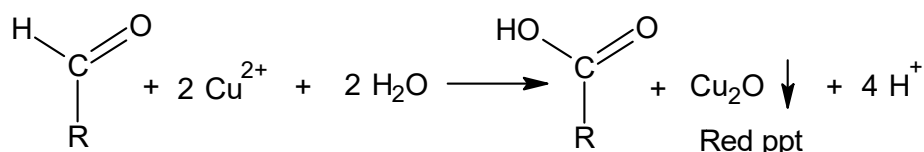
In the case of reducing sugars, the presence of alkali causes enolization especially at higher pH and temperature. This leads to a higher susceptibility to oxidation reactions that at neutral or acidic pH. These sugars, therefore, become potential agents capable of reducing certain mild oxidants such as copper(II) ions and Ag⁺ ions. Most commonly used reagents of this category for differentiating between reducing and non-reducing sugars are Fehling's test, and Benedict's test. In Barfoed's test mild acidic conditions are used (copper (II) acetate and acetic

Some disaccharides have free anomeric carbon and also reducing sugars like lactose. While other disaccharides such as sucrose are non-reducing sugars and will not react with copper(II) solution.

Large polymer of glucose such as starch, are not reducing sugars, since the concentration of hemiacetal group is very low. With this brief discussion on reducing and non-reducing sugars, now we take up the chemistry of the commonly used reagents for distinguishing them.

Fehling's and Benedict's Test

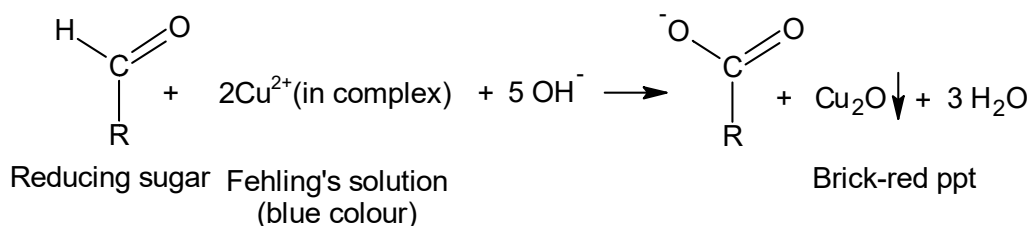
Both Fehling and Benedict's test are used to differentiate between reducing and non-reducing sugars. In these tests, Cu²⁺ is reduced to Cu⁺ and the reducing sugar is oxidized to a carboxylic acid:



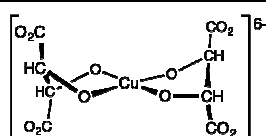
The appearance of copper(I) oxide, which is brick red, confirms the sugar to be a reducing sugar. Non-reducing sugars do not react. In both the reagent alkaline medium is used, therefore, to improve the solubility of Copper(II) ions and to prevent precipitation of copper ion as copper hydroxide, complexing agents are used. For Fehling's solution we use tartrate ions and for Benedict's solution we use citrate ions.

Fehling's solution is always prepared fresh in the laboratory. It is made from 2 separate solutions viz., Fehling's A and Fehling's B. The former is a blue colour solution of copper sulphate pentahydrate (CuSO₄·5H₂O), while the latter is a clear and colorless solution of aqueous potassium sodium tartrate (Rochelle salt) and a strong alkali (NaOH). In the final mixture, aqueous tartrate ions chelate to Cu²⁺ ions as bidentate ligand giving the bistartratocuprate(II) complex as shown in figure in margin. The tartrate ions, by complexing with copper prevent the formation of Cu(OH)₂ from the reaction of CuSO₄·5H₂O and NaOH present in the solution.

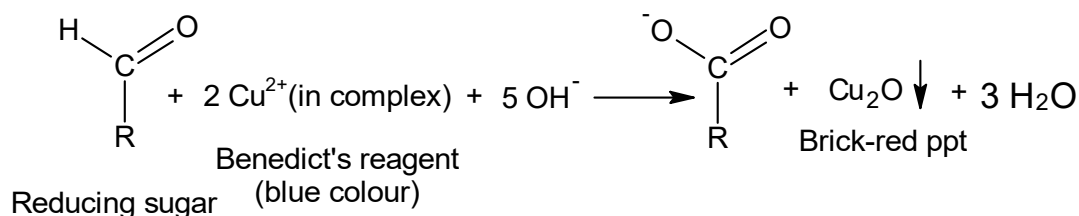
The net reaction between an aldose sugar and the copper(II) ions of Fehling's solution may be written as:



Benedict's solution is a bright blue solution prepared by mixing copper sulphate CuSO₄·5H₂O, sodium citrate (Na₃C₆H₅O₇), and sodium carbonate (Na₂CO₃) in distilled water. Citrate provided better stability for copper(II) ions. Net reaction may be written as

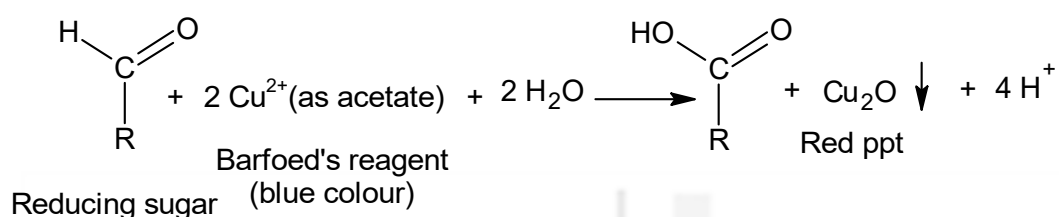


**Bistartratocuprate
(II) complex**



Barfoed's Test

Barfoed's reagent, a mixture of ethanoic (acetic) acid and copper(II) acetate. This also differentiates between reducing and non-reducing sugars, but because it is more sensitive than the Benedict's test, it can be used to differentiate between reducing monosaccharides and reducing disaccharides. The reduction of Cu^{2+} to copper(I) oxide occurs more rapidly for monosaccharides than for disaccharides. The rate of appearance of the red precipitate can distinguish reducing monosaccharides from reducing disaccharides. Net reaction may be written as:



11.3 REQUIREMENTS

Apparatus		Chemicals
Test tubs	6	Fehling's solution A and B
Water bath	1	Benedict's solution
Or 500 cm ³ Beaker	1	
Test tube stand	1	Barfoed's solution
Test tube holder	1	Sugars: glucose, fructose, lactose, maltose, sucrose, starch.

Solution Provided

Fehling's:

Fehlin's Solution A: Dissolve 7 g of $\text{Cu}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ in water and make up to 100 cm³.

Fehling's Solution B: 35 gm of sodium pottassium tartarate and 10 gm of NaOH Dissolved in 100 cm³ of distilled water.

Benedict's solution:

100 cm³ Benedict's solution can be prepared from 10 g of anhydrous sodium carbonate, 17 g of sodium citrate and 1.7 g of copper(II) sulphate pentahydrate.

Barfoed's solution:

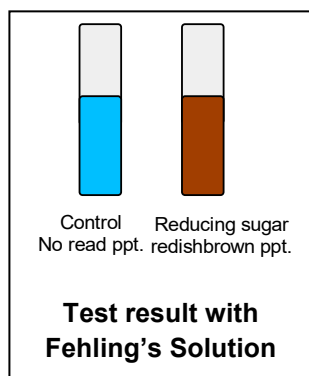
Dissolve 13.3 g of copper acetate in 200 ml of distilled water and add 1.8 ml of glacial acetic acid to it.

5 % Sugar solution of each glucose, fructose, lactose, maltose, sucrose, starch, unknown sugar solution:

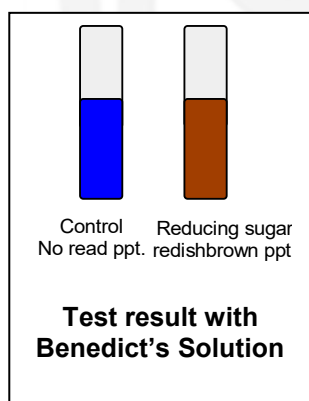
Dissolve 5 g of each sugar in 100 cm³ distilled water.

11.4 PROCEDURE**Procedure of Fehling's test:**

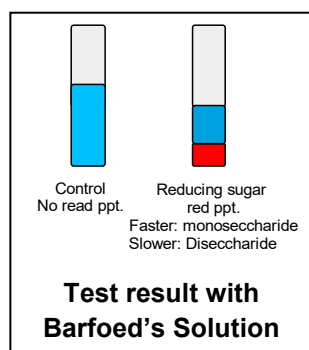
1. Take 1 cm³ of test sample in dry test tube.
2. Take 1 cm³ of distilled water in another test tube as control.
3. Take 2 cm³ each Fehling's solution A and B in a test tube and add 2 cm³ of this mixture to the sugar sample and distilled water.
4. Keep both sample solution and control test tubes in boiling water bath for 3-5 minutes.
5. Observe for the development of radish brown/brick red precipitate.
6. Record the observations in the Observation Table I.
7. Repeat the experiments for other sugar samples and unknown sugar solution.

**Procedure of Benedict's test:**

1. Take 1 cm³ of test sample in dry test tube.
2. Take 2 cm³ of distilled water in another tube as control.
3. Add 2 cm³ of Barfoed's reagent to both the tubes.
4. Keep both the test tubes in boiling water bath for 3-5 minutes.
5. Observe for colour change in the solution of test tubes or precipitation formation for the development of radish brown/brick red precipitate..
6. Record the observations in the Observation Table II.
7. Repeat the experiments for other sugar samples and unknown.

**Procedure of Barfoed's test:**

1. Take 1 cm³ of test sample in dry test tube.
2. Take 1 cm³ of distilled water in another tube as control.
3. Add 3-4 cm³ of Barfoed's reagent to both the tubes.
4. Keep both the test tubes in boiling water bath for some time.
5. Look for the development of brick red precipitate.



6. To check whether the red precipitates or red color are formed or not, remove the test tube regularly from the bath. Put the test tube again and again in the bath. Note the time taken to develop the color.
7. Record the observations in the Observation Table III.
8. Repeat the experiments for other sugar samples and unknown.

11.5 OBSERVATION

Observation Table I
Test with Fehling's Solution

	Material	Colour of mixture	Reducing/non-reducing sugar
1	Control	Blue	--
2	Glucose		
3	Fructose		
4	Maltose		
5	Sucrose		
6	Lactose		
7	Starch solution		
8	Unknown		

As compare to the original sugar solution, Barfoed's reagent should be added in more quantity. This ensures the good reaction and a positive result will be more observable.

It is very necessary to keep track of the time. Make sure that you have a digital stop watch beside you while you are performing the test. Timing is everything in this test and you might lose your experiment if not taken care of the time.

Observation Table II
Test with Benedict's Solution

	Material	Colour of mixture	Reducing/non-reducing sugar
1	Control	Blue	
2	Glucose		
3	Fructose		
4	Maltose		
5	Sucrose		
6	Lactose		
7	Starch solution		
8	Unknown		

As compare to the original sugar solution, Barfoed's reagent should be added in more quantity. This ensures the good reaction and a positive result will be detected easily.

It is very necessary to keep track of the time. Make sure that you have a continually watch time while you are performing the test. Timing is important criteria in this experiment.

Observation Table III
Test with Berfoed's Solution

Material		Colour of mixture	Reducing/ nonreducing sugar	Monosaccharide/ Disaccharide
1	Control	Blue		
2	Glucose			
3	Fructose			
4	Maltose			
5	Sucrose			
6	Lactose			
7	Unknown			

11.6 RESULT

Identification of Unknown carbohydrate:

Test reagent

	Observation	Conclusion
1. Fehling's solution:	_____	_____
2. Benedict's solution:	_____	_____
3. Berfoed's Solution:	_____	_____

EXPERIMENT 12

Determination of the Concentration of Glycine Solution by Formylation Method

Structure

12.1	Introduction	12.4	Requirements
	Expected Learning Outcomes	12.5	Procedure
12.2	An Overview of the Analysis of Organic Compounds	12.6	Observations
		12.7	Calculations
12.3	Principle	12.7	Results

12.1 INTRODUCTION

In Courses BCHCL132 and BCHCL 134, you were introduced to the various techniques for quantitative and qualitative analysis. We have described different methods which are used in quantitative analysis i.e., titrimetric methods refer to measurements of a volume, and physiochemical (instrumental) methods are based on the measurement of some physical or chemical property. In this experiment, we will use titrimetric methods for organic quantitative analysis. In the section 12.2 of this experiment; we will first give you an overview of the organic analysis. This will give you an idea as to how organic quantitative analyses are useful in organic analysis. After that you will be introduced to the actual experiment in which you will use formylation methods for the determination of the concentration of glycine solution.

Expected Learning Outcomes

After performing the experiment given, you should be able to:

- ❖ describe the significance of organic quantitative analysis in organic analysis;
- ❖ determine the amount of glycine in the given sample;

- ❖ describe formylation reaction; and
- ❖ perform acid-base titration using standard alkali.

12.2 AN OVERVIEW OF THE ANALYSIS OF ORGANIC COMPOUNDS

As we have discussed earlier, the following is an outline of the methods used in the study of organic compounds.

(a) Separation and Purification: Before the properties and structure of an organic compounds can be completely investigated the compound must be pure. Common methods of separation and purification are:

1. Extraction
2. Crystallisation
3. Sublimation
4. Distillation
5. Chromatography

There are various criteria for determining purity. The most common one for solids is m.p; for liquids, b.p. and more recently infra-red (IR) spectrum has been used as a test for purity. In all cases, the process of purification is repeated until the physical constant or spectrum remains unchanged. Methods of separation and purification of organic compounds, and of testing their purity, are described in the courses BCHCL 134.

(b) After getting pure organic compound, next step is identification and characterisation of the structure of the compound. This can be achieved by organic qualitative and organic quantitative analysis.

Organic Qualitative Analysis:

Qualitative analysis gives information about the presence of elements such as nitrogen, sulphur or the halogens, and functional groups such as $-\text{OH}$, $-\text{CO}$, $-\text{COOH}$, $-\text{NH}_2$ etc. Following steps are involved in this process.

- i) Physical examination
- ii) Elemental analysis
- iii) Solubility test
- iv) Determination of physical constant
- v) Functional group analysis
- vi) Preparation of derivation.

We have already discussed these steps in quite detail in the BCHCL 134 and earlier experiments of this course.

Organic Quantitative Analysis

Having known the constituent elements and the functional groups present in an organic compound, the next important step in its analysis involves quantitative analysis. Permitting the calculations of an empirical formula, this gives the atomic ratio of the elements present. Determination of the relative

Experiment 13 Determination of the Concentration of Glycine Solution by Formylation Method

molecular mass permits the assignment of a definite molecular formula that expresses the actual number of atoms of each element present in the compound. Further quantitative functional group analysis gives the information about the number of functional group present in the substance.

There are two approaches to this. One is so far discussed methods of analysis, traditional, it depends on chemical reactions. The modern method involving spectrometry, is discussed in the course of higher semester course. Spectrometric methods are used extensively today because they are faster and are capable of dealing with small amount of compounds with more complex structures. Although the traditional methods now are seldom used alone, they are described in lab courses of our B.Sc. programme for a number of reasons. On occasion, parts of the traditional schemes are still quite useful. Also, the required technique strongly reinforces fundamental chemical and physical principles and exposes the student initially to make the right chemical judgments, an essential skill for productive research. The time invested in learning how to interpret chemical and physical behaviour will be repaid many times over in future work. Last reason is that most of our laboratories are not equipped with the modern instrumentations.

Now we will concentrate on the organic quantitative analysis.

The organic quantitative analysis consists of a series of steps that not only helps to establish the identity of the compounds but also provides methods for the determination of amounts or concentration of constituents. There are two main types of quantitative analysis which are generally carried out for organic compound:

i) Quantitative elemental analysis:

This is carried out to find out the relative numbers of the different kinds of atoms, that is to determine the empirical formula. This in turn combined with the molecular formula weight shows the actual numbers of the different kinds of atom, that is, gives us the molecular formula. In recent years it has become possible to find the molecular formulae of some compounds directly by high resolution mass spectrometry.

ii) Quantitative functional group analysis: This is carried out to find the relative number of the different kinds of functional groups.

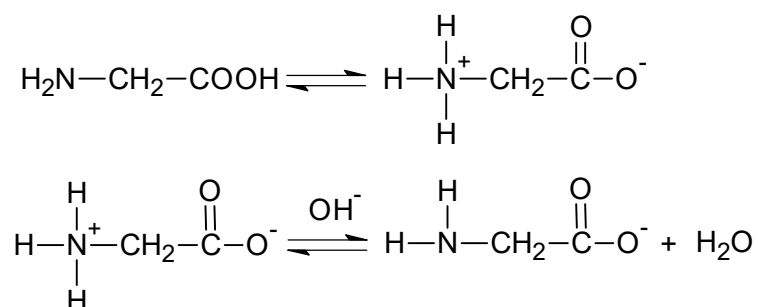
Quantitative Functional Group Analysis:

The quantitative estimation of the functional groups is based on the stoichiometric equations of the reactions such as neutralisation, acetylation, reduction, oxidation, addition, hydrolysis etc. Function group analysis not only helps to estimate functional groups present in a compound but also provides methods for the determination of amount or concentration of organic constituents. In this course, you will be introduced to one such experiment. As a representative example of quantitative analysis you will be estimating glycine in the given solution.

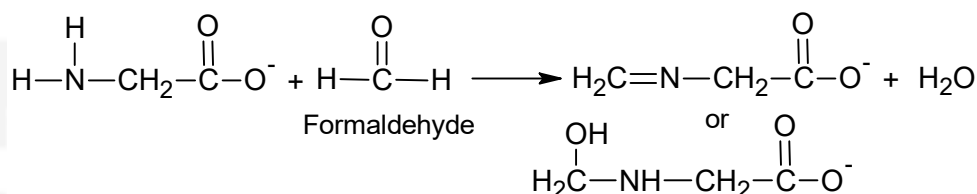
There are many titrimetric methods available for the determination of amino acids. Here we will estimate glycine by formylation method, which is also called Soronsen's method.

12.3 PRINCIPLE

Amino acids like glycine, alanine, etc. contain one amino group and one carboxylic group as part of their structures. These groups being of opposite nature neutralise one another intramolecularly and form internal salts called zwitter ions or dipolar ions. These ions are held together by electrostatic attraction. They are neutral but in presence of alkali the dissociation favours formation of acid ion.



The free amino group then undergoes condensation with formaldehyde to form mono and dimethyl derivatives. Thus, the formation of these condensation products greatly reduces the basic character of amino group and the carboxylic group can readily be titrated with standard alkali.



12.4 REQUIREMENTS

Burette (50 cm ³)	1	Sodium hydroxide
Pipette (25 cm ³)	1	Formalin solution
Vol. flask (250 cm ³)	1	Phenolphthalein indicator
Conical flask (250 cm ³)	1	
Weighing bottle	1	
Wash-bottle for distilled water	1	
Funnel (small)	1	
Test-tube	1	
Burette stand	1	

Solution Provided

- Sodium hydroxide solution (0.1 M): Dissolve 2 g of sodium hydroxide in a 250 cm³ volumetric flask and make up to the mark with distilled water.
- Neutral 40% formalin solution: Take 50 cm³ of 40% formalin solution in a 250 cm³ conical flask and add 8-10 drops of phenolphthalein indicator. To

Experiment 13 **Determination of the Concentration of Glycine Solution by Formylation Method**

it add carefully from a burette a dilute solution of sodium hydroxide (0.1 M), till the solution is just faintly pink.

- iii) Phenolphthalein indicator: Dissolve 1.0 g of phenolphthalein in 100 cm³ of ethanol and then dilute with 100 cm³ of water.

12.5 PROCEDURE

- i) Preparation of Standard solution of glycine: Weigh accurately 0.8 g of glycine and transfer to a 100 cm³ volumetric flask and make up to the mark with distilled water.
- ii) Titration with standard solution: Take 20 cm³ of standard glycine solution in a 250 cm³ conical flask and add 3-4 drops of phenolphthalein indicator. Add dilute sodium hydroxide solution (0.1 M) taken in burette drop by drop to it until a pink colour is just obtained. Now add 10 cm³ of neutral formalin solution to it. The pink colour of the solution immediately disappears. Continue adding sodium hydroxide slowly till pink colour is restored. Note the volume of sodium hydroxide used and repeat the experiment until two concordant readings are obtained. Record the observations in the Observation Table I.
- iii) Titration with unknown glycine solution: Perform the titration as described above for 20 cm³ unknown glycine solution and note the volume of sodium hydroxide used in this titration. Record the observations in Observation Table II.

12.6 OBSERVATIONS

Mass of the weighing bottle = m_1 = ...g

Mass of the bottle + glycine = m_2 = ...g

Mass of the bottle (after transferring the compound) = m_3 = ...g

Mass of glycine transferred $m_2 - m_3 = m = \dots\dots$ g

Observation Table I
Standard Glycine Solution vs. Sodium Hydroxide Solution

Sl. No.	Volume of glycine solution in cm ³	Burette reading		Volume of sodium hydroxide solution in cm ³ (Final - Initial)
		Initial	Final	
1	20			
2	20			
3	20			

Observation Table II
Unknown Glycine solution vs. Sodium hydroxide

Sl. No.	Volume of glycine solution in cm ³	Burette reading		Volume of sodium hydroxide solution in cm ³ (Final - Initial)
		Initial	Final	
1	20			
2	20			
3	20			

12.7 CALCULATIONS

Mass of glycine dissolved in $100 \text{ cm}^3 = m \text{ g}$

The volume of sodium hydroxide solution used for 20 cm^3 of standard glycine solution $= V_1 \text{ cm}^3$

The volume of sodium hydroxide solution used for 20 cm^3 of unknown glycine $= V_2 \text{ cm}^3$

Volume of glycine solution taken for titration $= 20 \text{ cm}^3$

$V_1 \text{ cm}^3$ of sodium hydroxide solution $= 20 \text{ cm}^3$ of standard glycine solution

$$= \frac{m}{5} \text{ g of glycine}$$

$V_2 \text{ cm}^3$ of sodium hydroxide solution $= 20 \text{ cm}^3$ of unknown glycine solution

$$= \frac{m}{5} \times \frac{V_2}{V_1} \text{ g of glycine}$$

Thus strength the strength of the unknown glycine solution

$$= \frac{m}{5} \times \frac{V_2}{V_1} \times \frac{1000}{20} \text{ g dm}^{-3}$$

$$= \frac{m \times V_2 \times 10}{V_1} \text{ g dm}^{-3}$$

12.8 RESULT

The amount of glycine in the given solution $= \text{.....g.}$

The strength of the unknown glycine solution $= \text{.....g dm}^{-3}.$

EXPERIMENT 13

ACTION OF SALIVARY AMYLASE ON STARCH AND EFFECT OF TEMPERATURE ON THE ACTION OF SALIVARY AMYLASE ON STARCH

Structure

13.1	Introduction	13.4	Procedure
	Expected Learning Outcomes	13.5	Observations
13.2	Principle	13.6	Results
13.3	Requirements		

13.1 INTRODUCTION

In Block 4 of BCHCT-135, we have discussed the chemistry of proteins. We have also mentioned that enzymes are protein catalysts and they act as catalysts for biological reactions. Enzymes also, like other catalysts, increase the rate of reactions without being used up themselves. They do this by lowering the activation energy of a reaction. As enzymes are proteins, they can be denatured on variation of temperature and pH. Most of enzymes have optimum activity at a static pH and at body temperature.

In this experiment we will take up the salivary amylase enzyme to study the effect of temperature. This enzyme is responsible for hydrolysis of starch.

Expected Learning Outcomes

After performing the experiment given, you should be able to:

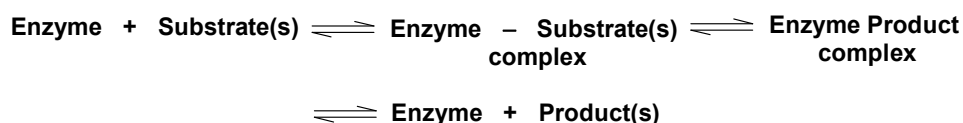
- ❖ explain various factors which influence enzyme activity;
- ❖ describe the reaction of amylase enzyme with starch;

The major enzyme found on human saliva is α -amylase. Healthy people produce about 1.5 dm^3 of saliva per day.

- ❖ describe how temperature affects the hydrolysis reaction of starch and amylase, and;
- ❖ demonstrate the effect of temperature on the activity of salivary amylase using indicator method.

13.2 PRINCIPLE

The ability of enzymes to function as catalyst depends on its shape of the protein. Recall that all proteins have a specific shape and they have many different types of active binding sites. Because of these characteristics, they are very specific – They only act on one substrate or one class of related substrates. A typical enzyme catalyzed reaction can be represented as



Factors Influencing Enzyme Activity

Enzymes are among the fastest catalysts known. The ability of an enzyme to convert substrate into product is called enzyme activity.

Enzyme activity is not necessarily constant – there can be a number of factors that influence their activity. Here, we consider some common factors that can influence enzyme activity.

1. Effect of Enzyme Concentration

In enzyme catalysed reaction each enzyme molecule has one more active binding site for conversion of substrate molecule to product molecules. Thus the more enzyme is available, the quickly substrate can be converted into product. If we consider that all these factors are constant, in general as enzyme concentration increases, there is a proportional increases in reaction rate (see Fig. 13.1).

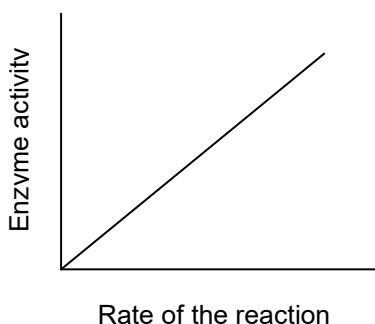


Fig. 13.1: Plot showing the mathematical relationship between enzyme concentration and enzyme activity.

2. Effect of pH

Each enzyme has an optimum pH. Above or below an enzyme optimum pH, its activity is lower. Mostly in human body function optimally at pH between 7-8, some enzymes, such as those of our digestive system, may have optimal pH at very acidic (pH 2) or very alkaline (pH > 8). This

effect is actually due to the change in protein structure of the enzyme at different pH values similar to colour indicators in acid base titrations. In other words, enzymes become denatured when exposed to different acidic condition than its optimum pH conditions. The variation in the rate of enzyme – catalysed reaction with pH is shown Fig. 13.2.

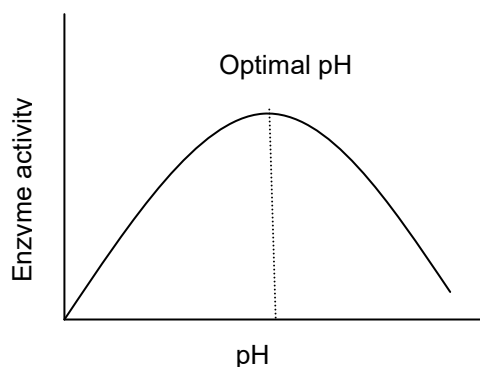


Fig. 13.2: Plot showing variation in the rate of enzyme – catalysed reaction with pH.

Effect of Temperature

In general, rate of chemical reaction both catalysed and non-catalysed reactions, tend to increase as temperature increases. This is because at higher temperature, in reaction mixture molecules tend to be moving more quickly than at lower temperature and thus, there is more tendency of collision with one another with greater energy. However, enzyme-catalysed reactions become slower or stop if the temperature becomes too high (above 70°C), because enzymes become denatured at high temperature. Fig. 13.3 illustrates the effect of temperature on enzymatic activity.

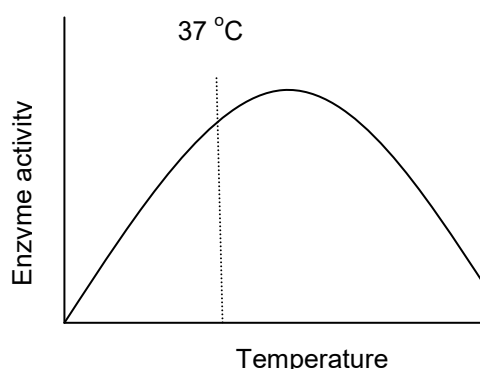


Fig. 13.3: Plot showing the relationship between the rate of enzyme catalysed reaction and temperature.

There are few more other factors which also influence the enzyme activity such as effect of substrate concentration, effect of co-factor/coenzyme concentration and inhibitors etc., At this stage, we are not going in details of these factors.

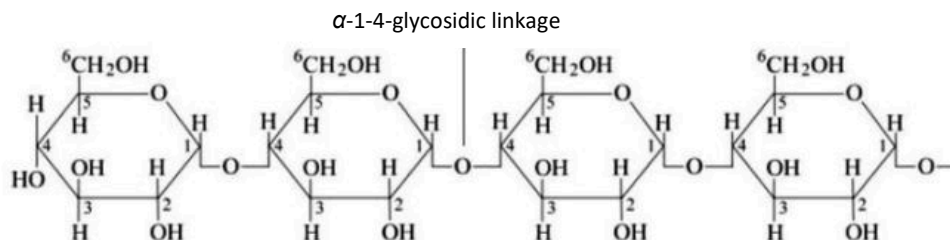
Reaction between starch and salivary amylase

Our saliva contains an enzyme called salivary amylase. This reacts with the starch component of our food and hydrolyses it mainly into smaller saccharide

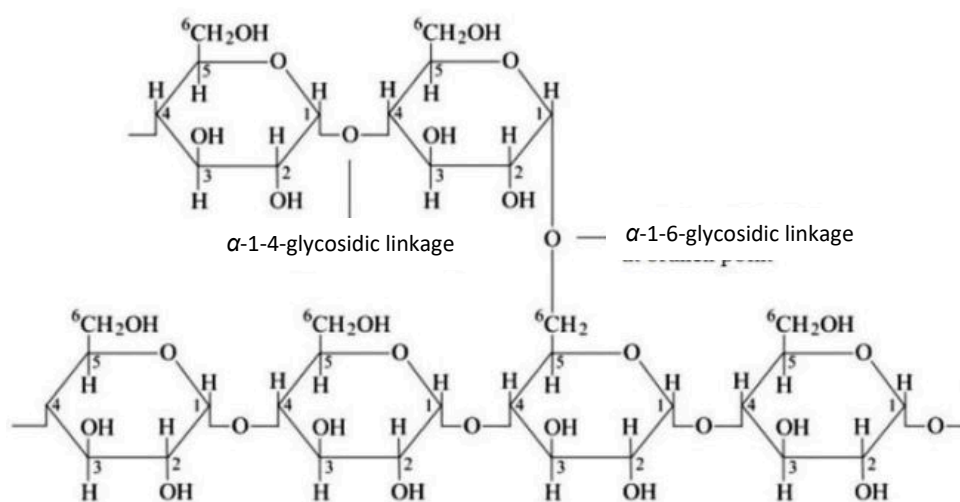
The secondary, tertiary, and quaternary structures of proteins all rely on relatively weak non-covalent bonds, for example hydrogen bonds, van der Waals forces, and ionic bonds to link different regions of the protein together. Increased temperature causes random movement in different regions of the protein, thus destabilising these weak bonds and causing a change in the shape of the protein (denaturation). If large numbers of these weak bonds are broken, the shape of the active site will begin to distort and the enzyme will lose its ability to bind the substrate and catalyse the reaction.

units such as maltose. Complete hydrolysis of starch into glucose occurs in the small intestine in the presence of a very active pancreatic enzyme.

Starch is a polysaccharide made of two compounds, amylose and amylopectin. These two polymers consist of glucose monomers that are joined by glycosidic bonds. The enzyme amylase catalysed the breakdown of starch into disaccharides and finally to glucose molecules.

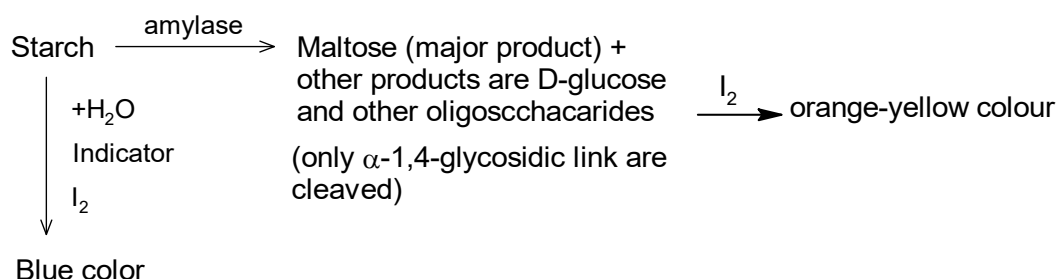


Amylose



Amylopectin

An easy way to track the rate of reaction catalysed by enzyme is by means of an indicator. For this reaction under study, iodine solution is used as a starch indicator and can be used to track the digestion of starch by amylase. Iodine solution is typically orange-yellow in color, and in the presence of starch it becomes blue-black. Iodine molecules in aqueous solution are arranged in random order. However, when starch is added to the iodine solution, it binds to iodine and organises it in non-random arrangement. Iodine in this non-random arrangement imposed by the starch chain is blue. When starch gets broken down into maltose or glucose, it can no longer arrange iodine non-randomly. So the solution turns yellowish again. The reaction of I_2 and starch with amylase enzyme can be represented as follows:



13.3 REQUIREMENTS

1.	Stop watch	-	1
2.	Test tubes (or 6 test tubes + 3 spot plates)	-	20
3.	Droppers	-	3
4.	Constant temperature water bath	-	2
5.	Thermometers	-	3
6.	Beakers	-	3

Solution Provided

1. 1% Starch solution:
 - i) Place 90 cm³ of distilled water in a 250 cm³ beaker and bring to boiling on a hot plate.
 - ii) Make a smooth paste with 1 g of soluble starch in 10 cm³ of distilled water.
 - iii) Once the water is boiling, carefully remove the beaker containing the boiling water from the hot plate. Pour the starch paste into the boiling water and stir until all of the starch is dissolved. The resulting solution may be somewhat cloudy.
 - iv) Allow the starch solution to cool to room temperature before use.
2. 1% NaCl Solution: 1 g of NaCl in 100 cm³ of distilled water.
3. Iodine solution: Dissolve 1 g of iodine crystals and 2 g of potassium iodide in 100 cm³ of distilled water.

13.4 PROCEDURE

1. If your laboratory has two multi holes water baths, set one of them at 37°C and other can be set close to 100°C (boiling temperature), and every one of you will use these water baths turn by turn. You will also need one more low temperature water bath. You can make your own using a 250 cm³ beaker or copper water bath; fill it with some water add some ice to the water and maintain temperature between 0-5°C. If you Lab does not have large water baths for higher temperatures, then you have to make own water bath using 250 cm³ beaker or copper bath and regulate their temperature using Bunsen burner/hot plate.
2. Take 15 cm³ 1% starch solution and 3 cm³ NaCl solution in a beaker and also take about 50 cm³ of iodine solution in another beaker.
3. You can prepare your own fresh amylase solution by collecting about 1-2 cm³ of your own saliva in a small beaker (You will need to spit politely into the beaker). Take 1 cm³ of saliva, add 19 cm³ of water to saliva and

mix well. If a commercial amylase solution is available, you may use it instead of your own saliva.

4. Take three sets of test tubes (6 test tubes in each set) in three separate test tube stands. Add 1 cm³ of iodine solution in each test tube. Mark each test tube stands as A (for 5 °C), B (for 37 °C), C (for 100 °C) and also mark test tubes of each set as 0, 1, 2, 3....
 5. Take another three test tubes and mark them as Experiment Tube A, Experiment Tube B and Experiment Tube C and add 5 cm³ mixture of starch solution + NaCl solution to each test tube.
 6. Now transfer Experiment Tube A into water bath/beaker containing ice maintained at 5°C. Transfer the second Experiment Tube B into water bath set at 37°C and the third Experiment Tube C into boiling water bath maintained at 100°C.
- Keep the tubes in their baths for 10 minutes to allow them to reach the temperature of their baths.
7. Read and record the temperature of all water baths. Add 1 cm³ of saliva solution into each test tube marked as Experiment Tube A, B and C.
 8. Immediately, using a dropper, take few drops from tube A and transfer them into tube 0 of A series of test tubes having iodine solution.
 9. Similarly, using fresh droppers do the same procedure for Experiment Tube B and Experiment Tube C and transfer the few drops of solution into B and C series of test tubes having iodine solution. Note the first addition of reaction mixture to iodine as zero minute reading.
 10. At intervals of every 2 minutes keep on repeating the above step and note the change in colour of iodine solution. Continue this till the colour of iodine does not change.
 11. Note the time taken for different experimental tubes till they do not give any colour with iodine.

13.5 OBSERVATIONS

Table

Time/ minutes	Reaction with Iodine from Experiment Tube A	Reaction with Iodine from Experiment Tube B	Reaction with Iodine from Experiment Tube C
0			
2			
4			
6			
8			

13.6 Result

Result: To digest completely 5 cm³ of 1% starch solution, 1 cm³ of enzyme takes minutes at 5°C minutes at 37°C and minutes at 70°C.

