EXPERIMENT 1
SEPARATION OF A MIXTURE OF BENZOIC ACID, 2- NAPHTHOL AND 1,4-DIMETH-OXYBENZENE BY SOLVENT EXTRACTION AND IDENTIFICATION OF THEIR FUNCTIONAL GROUPS

Structure

1.1 INTRODUCTION

The mixture mentioned in the experiment title contains two acidic and one neutral compound.The pK_a values of the acidic compounds viz., benzoic acid and 2 naphthol are 4.17 and 9.51 respectively. Benzoic acid is a stronger acid while 2–naphthol is weakly acidic.Seperation of this mixture will require the extraction of the acidic components one by one. You will be using the technique of solvent extraction for this purpose.Since this technique is new to you we will give a brief account of this in the next section before describing the experiment.

Objectives

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

- separate a mixture of a neutral and an acidic organic compound by solvent extraction technique,
- identify the functional groups of the separated compounds, and
- describe the theory behind the above.

1.2 SOLVENT EXTRACTION

You are aware that most of the organic reactions normally do not go to completion. The reaction mixture contains unreacted reactants and unwanted side products besides the desired product. In such a situation one of the products (wanted or unwanted) may need to be separated. One of the methods which can be used to perform such a separation is called as **solvent extraction**. This method is based on the principle of phase distribution . It exploits the differential solubility of a given solute in two immiscible solvents to separate it from a given mixture. Let us briefly understand the technique.

Suppose a substance 'X' has different solubilities in two immiscible solvents. If we take a solution of the substance in any of the solvents and shake with the second solvent then it distributes itself in two solvents depending on its solubility in these.For example acetanilide is soluble in ether as well as in water. If we take its solution in water and shake with ether then part of acetanilide goes to the ether layer. The two layers can be separated and the ether layer can be evaporated to get acetanilide. We can repeat the process a number of times whereby more and more of acetanilide would come to ether and eventually all the acetanilide from aqueous layer would get 'extracted'.

The extracting efficiency of the solvent depends on the distribution coefficient of the solute in the two solvents. Distribution coefficient is defined as the ratio of the concentration of the solute in two solvents.

$$K = \frac{\text{conentration of the solute in solvent 'A'}}{\text{concentration of the solute in solvent 'B'}}$$

$$= \frac{\text{solubility of the solute in solvent 'A'}}{\text{solubility of the solute insolvent 'B'}}$$

This definition of distribution coefficient holds only in the solute remains in same molecular state in both the solvents. If, however, the solute associates or dissociates in one or both the solvents the defining equation has to be modified. You can referto any standard text on physical chemistry for these equations.

It is obvious that higher the value of *'K'* higher is the extracting efficiency, i.e., in event of extraction more amount of the solute would be transferred. Thereby smaller volume of extracting solvent would be required. A good extracting solvent should have the following properties.

It should be a good solvent for the substance being extracted i.e., solute should have high solubility in this solvent,

- it should have low boiling point so that the extracted substance can be recovered easily,
- it should not be expensive,
- it should not react with the solute or the other solvent, and
- of course, it should be immiscible with the other solvent.

In this and the next experiment you will be performing extraction of acidic and basic compounds respectively from their mixtures with neutral substances.To accomplish such an extraction the mixture is normally taken in an organic solvent and is shaken with an aqueous solution of a base or an acid. This process is called as **acid base extraction.** Let us take an example of separation of an acidic component from the rest in a mixture to understand the process of acid base extraction. When we shake such a mixture with an aqueous solution of a base the acidic compound gets extracted into aqueous phase as its salt. The extraction process can be visualised as follows.

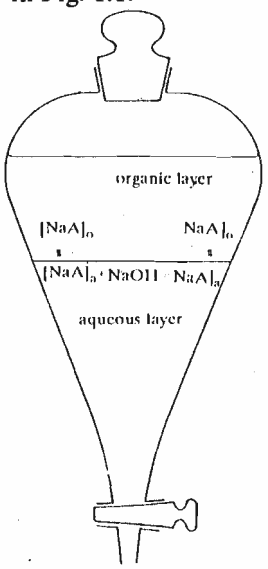
When we mix the organic solvent (containing acid) with aqueous solution of the base, the acid distributes itself into organic and aqueous layer depending on its distribution coefficient. The base present in aqueous layer immediately converts the acid into its salt, Eq. 1.1.

$$RCOOH + MOH \longrightarrow RCOOM + H_2O \qquad \ldots (1.1)$$

The extent of conversion depends on the strength of the base. In any case the concentration of free acid in aqueous layer becomes very small. As a consequence, to maintain the distribution coefficient more acid comes into the aqueous phase and gets converted into the salt. This process continues till the base is able to perform this conversion completely. The salt formed in this process also distributes itself into the two solvents. Due to very large solubility of the salt in aqueous phase as compared to organic phase, most of the salt stays in the aqueous layer. That is, a very little amount of acid goes back into organic layer as salt.

The net result of these processes brings most of the acid into aqueous phase. The amount of acid which comes to the aqueous layer depends on the amount and nature of the base (weak or strong). If sufficient quantity of appropriate base is present then

practically all the acid from the organic layer comes into aqueous layer and we get a good (!) extraction. The equilibria involved in these processes are represented schematically in Fig. 1.1.



$[HA]_a$: Concentration of acid HA, in aqueous layer

$[HA]_o$: Concentration of acid HA, in organic layer

$[NaA]_a$: Concentration of the salt NaA in aqueous layer

$[NaA]_o$: Concentration of the salt NaA in organic layer

Organic soluents like chloroform and carbon tetrachs oride. form lower layer with water while, solvents like either, and petrol form upper layer with water in seporatory funnel.

Separation of a Mixture of Benzoic Acid, 2-Naphthol and 1, 4–Dimethoxy-henzene by solvent extraction and Identification of their Functgional groups.

Fig. 1.1. Schematic representation of the eguilibria involved in the extraction of an acidic compound from organic to aqueous layers

The appropriate base refers to the one which can effectively convert the acidic compound into its salt. As a rule of the thumb a basic solution whose pH is at least 4 pH units more then the pK_a of the acid to be extracted can afford an almost complete extraction. For example benzoic acid (pK_a 4) can be extracted quite effectively by a 5 % aqueous solution of sodium bicarbonate (pH ~ 11). The approximate pK_as of common organic acids or bases and the pH of the common extracting solutions are given in Table 1.1 and 1.2 respectively. These would be of help to you in devising the extraction strategy to separate any other mixture.

pK_a, as you know is an index of the strength of an acid. It is defined as –log K_a where K_a is dissociation constant of the acid. Higher the pK_a valuweaker the acid.

Table 1.1 : Approximate pK_a values of some common acidic/basic compounds

Class of compound	Approximate pK_a	Examples
Mineral acid	<1	HCl,HNO_3
Aromatic carboxylic acid	4	benzoic acid
Aliphatic carboxylic acid	5	acetic acid, propanoic acid
Anilines	5	aniline, toluidine
Pyridines	6	pyridine
Phenols	10	1–naphthol,phenol 2–naphthol
Aliphatic amines	11	methyl amine, ethylamine

Table 1.2 : Approximate pH values of the solutions (5–10 % by weight) commonly used for acid–base extraction

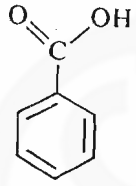
Compounds	Approximate
pH HCl; H_2SO_4	0
Acetic acid	3
$NaHCO_3$	8
Na_2CO_3, K_2CO_3	11
NaOH, KOH	14

Needless to say that the other species (which are not acidic) would stay in organic layer. Only a small portion would come into the aqueous layer depending on its solubility and distribution coefficient. These are removed in the event of acidification or crystallisation. Further, you may be wondering that we wanted to separate acid and have landed up with a solution containing salt of the acid. Don't worry the acid can be recovered quite easily by acidifying the solution with mineral acid (pH = 1).

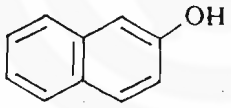
Similarly we can understand the extraction of a basic compound say an amine from an organic solvent by using an aqueous solution of an acid. Again as a rule of the thumb the pH of the extracting acid solution should be at least 4 pH units away from the pK_a of the conjugate acid of the base.For example an aliphatic primary amine (pK_a of conjugated acid, RNH = 11 can be effectively extracted by a 5–10 % solution of acetic acid (pK_a =3) . The acetate salt so obtained can be converted back to the amine by using aqueous solution of sodium hydroxide.

Having understood the concept of solvent extraction let us now discuss about the separation of mixture given in the experiment title.

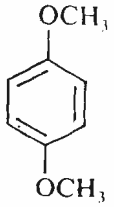
1.3 PRINCIPLE



Benzoic acid
pK_a 4.17

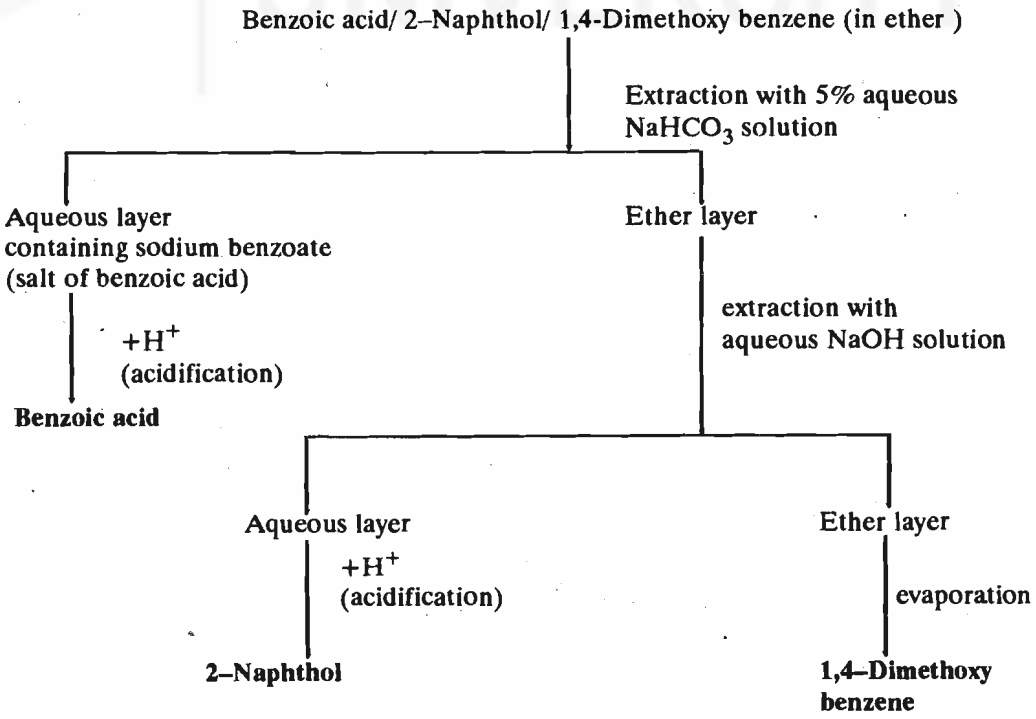


2-Naphthol
pK_a 9.5



p-Dimethoxybenzene
(Hydroquinone dimethyl ether)

You would recall from above that we need a basic solution to extract an acid into aqueous medium whose pH is at least 4 pH units away from the pK_a of the acid.We can see that to extract benzoic acid (pK_a 4.17) we need a solution whose pH is atleast 8.2 while for extracting 2-naphthol (pK_a 9.51) the solution should have a pH of 13.5 or above. If we use 1 *M* sodium hydroxide(pH 13) as the extracting solution both the compounds will be extracted whereas if we use $NaHCO_3$ then only benzoic acid would be converted to its salt while 2–naphthol would stay as such. Therefore, to separate the mixture we perform two extractions. First we extract the mixture with $NaHCO_3$ (to separate benzoic acid)and then we use sodium hydroxide to separate naphthol from 1,4- dimethoxybenzene. This strategy may be represented in the form of a flow chart as given below.



1.4 REQUIREMENTS

Apparatus		Chemicals
Separatory funnel (100 cm^3)	1	Solvent ether 40 cm^3
Beakers (100 cm^3)	2	Sodium sulphate
Conical flask (50cm^3	3	Sodium bicarbonate
Ring clamp	1	Sodium hydroxide
Funnel	1	Acetic acid
Filter paper		Sodium chloride

Solutions Provided

1. **10 % aqueous solution of sodium bicarbonate:** It can be prepared by dissolving 10g $NaHCO_3$ in 100 cm^3 water.
2. **10 % aqueous solution of sodium hydroxide:** This solution is prepared by dissolving 10g NaOH in 100 cm^3 of water.
3. **Saturated solution of NaCl :** It can be prepared by disiolving excess of NaOH in water.

1.5 PROCEDURE

The procedural instructions have been given below in sequential order.You are expected to go through the instructions carefully, and prepare a broad mental outline of the same.

1 Clean the separatory funnel first with soap water and then with plenty of water. Grease the stopcock to ensure its smooth movement.

Teflon stopcocks need not be greased

2 Close the stopcock and mount the separatory funnel in the ring support on an iron stand as shown in the margin. If the ring support is not available you may use a tripod stand for the purpose.

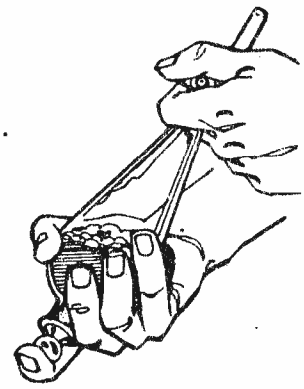
3. Weigh 3 g of the mixture on a rough weighing balance and dissolve it in about 30 cm^3 of solvent ether in a concial flask

4. Transfer the solution to the separatory funnel after ensuring that the stopcock is closed.Wash the conical flask with a little (5 cm^3) of ether and pour this also into the separatory funnel. This ensures complete transfer of the mixture.

If you don't release the pressure then the stopper may be blown off along with the spilling of the solution

5 Add about 20 cm^3 of 10 % aqueous solution of sodium bicarbonate (the extracting solution) to the funnel.Wet the stopper and place it on the funnel.This is done so as to avoid the spilling of organic solvent from the sides of the stopper.

6. Gently swirl the contents of the funnel to mix them. Release the pressure built up inside the flask. This pressure is due to the evaporation of highly volatile ether. Besides this, the neutralisation of the carboxylic acid group with sodium bicarbonate evolves CO_2 gas. To release the pressure, carefully turn the funnel upside down holding the stopper in place as shown in the margin and cautiously opening the stopcock.You would hear the sound of escaping vapours. Close the stopcock again and mix the contents well with repeated release of pressure.



Venting position, showing corret way of holding funnel for shaking and venting.

7. Allow the mixture to stand in the funnel (on the ring support) untill the two immiscible layers separate out.

8. Remove the stopper at the top and draw off the lower layer into a 50cm^3 conical flask labelled 'A'.

9. Add 10 cm^3 of 5 % aqueous solution of sodium hydroxide to the separatory funnel, shake the mixture thoroughly and allow the layers to separate after releasing any pressure built up.

10. Remove the stopper and draw off the lower layer in a concial flask labelled 'B'.

11. Put additional 5 cm^3 of H_2O in the funnel,shake the contents, allow the layers to separate and draw off the lower layer again in flask 'B'.

Commercial samples of anhydrous sodium sulphate might have absorbed some moisture. You may spread about 10 to 15g of the crystals of Na_2SO_4 in a petry dish or china dish and keep it in as oven maintained at 110°C for 45 hours. Allow it to come to room temperature and store in a dessicator.

12. Pour about 15 cm^3 of saturated aqueous solution of sodium chloride to the separatory funnel. Shake vigorously for about a minute and allow the layers to separate. Draw out the lower layer and discard it.

13. Pour the ether layer into a concial flask labelled as 'C' containing anhydrous sodium sulphate. This treatment removes the water which gets dissolved in ether and helps in its drying.

RECOVERY OF THE SEPARATED COMPOUNDS

By following the above procedure you will obtain three flasks containing three separate compounds.

Flask A : Benzoic acid as sodium benzoate in water.

Flask B : 2–naphthol as sodium salt in water.

Flask C : 1,4–Dimethoxybenzene in ether.

The desired compounds can be obtained as follows.

Be careful ! do not add acid in large amounts as it may lead to spilling of the solution.

Recovery of benzoic acid : You would recall from the introduction that pure benzoic acid can be recovered by acidification of its sodium salt. To accomplish this take flask 'A' containing the solution of sodium benzoate and to this carefully add dilute hydrochloric acid to that, dropwise with constant shaking of the flask. Continue addition till the solution becomes acidic. (check with the help of a pH paper). You will observe brisk effervescence, due to the neutralisation of excess bicarbonate.

When the pH of the solution reaches close to 4, you will see the separation of benzoic acid as a white solid. If you don't get it add a few drops more of hydrochloric acid and leave the solution for a few minutes. If you still don't get the crystals scratch the sides of the flask with the help of a glass rod and keep the solution in ice\icecold water. Filter the solid over buchner funnel or by ordinary gravity filteration. Dry the crystals in the folds of filter paper and weigh them.Report the amount of benzoic acid obtained. Save the sample for functional group determination.

Recovery of 2-Naphthol : 2-naphthol, is also recovered in the same way as benzoic acid, i.e., by acidifying with dilute hydrochloric acid solution. Here you would not be observing any effervescence as in this case the neutralisation involves reaction of sodium hydroxide and hydrochloric acid. After acidification (checked by pH paper) the solid is separated by filteration , dried in the folds of filtered paper and weighed. Report the amount of 2–naphthol obtained. Save the sample for functional group determination.

Recovery of 1,4-Dimethoxybenzene : Decant the etheral solution from flask 'C' into another flask. Put about 5 cm^3 of ether into flask 'C' and thoroughly rinse the flask along with the drying agent. Wait for a minute or two and mix this ether with the previous lot by carefully decanting it. Take care not to transfer the drying agent. Evaporate the ether on water bath or preferably distill it.

Caution : This step should be performed in a fuming cupboard. Do not inhale the vapours of ether. Remove all the flames in the lab before evaporating

You may add a few pieces of pumis stone or simply a glass tube to facilitate evaporation. Scratch the solid with a neat spatula and weight it.

Report the amount of 1,4 dimethoxy benzene obtained and save the sample for functional group determination.

1.5.1 Identification of Functional Groups

If the etheral solution is not dried properly you may not get the solid. Instead you would lend up with an oily mass.

As the separated compounds are known, we are providing the characteristic tests for the functional groups of the known compounds. For a generalised scheme for the identification of the functional groups in unknown compounds, you can consult Block 2 of CHE-08(L) course or the books listed in the bibliography.

Test for carboxylic acid group (–COOH) : Take about 0.1 g of benzoic acid (recovered above) in a test tube and add about 1 cm^3 of 5% aqueous solution of $NaHCO_3$ along the sides of the test tube. Observe carefully the point where this solution meets the solid compound. An effervescence along with evolution of a gas indicates the presence of a –COOH group.

$$RCOOH + NaHCO_3 \longrightarrow RCOONa + H_2O + CO_2.$$

You may repeat the same by taking larger amounts (0.4 g) of the compound and pass the evolved gas through a freshly prepared solution of lime with the help of a delivery tube. Milky colouration confirms the presence of a –COOH group.

$$Ca(OH)_2 + CO_2 \longrightarrow CaCO_3 + H_2O$$

Test for phenolic hydroxy group : The second acidic compound in this mixture, viz., 2-napthol bears a hydroxy group. The presence of such phenolic groups can be established by a number of tests. We are giving a few of them here. Generally more than one test must be made before concluding the presence of phenols. It is so because the nature and position of the substituents on a phenolic compound strongly influences the reaction shown by them.

1. **Ferric chloride test :** Phenolic compounds react with ferric chloride to produce coloured complexes. The test is performed as follows:

Dissolve 10-15 mg of the suspected compound (you will use 2- naphthol separated above) in about 1 cm^3 of water (or aqueous alcohol) and add 1-2 drops of aqueous ferric chloride solution. Development of an intense colouration (red, blue, purple or green) or formation of coloured precipitate indicates the presence of a phenolic group.

$$\text{Phenolic compound} + FeCl_3 \longrightarrow \text{Coloured complex}$$

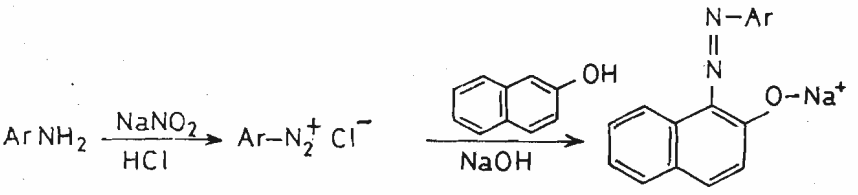
The colour may deepen after some time.

Incidentally 2-naphthol does not give colouration in aqueous solution but in aqueous alcoholic solution the test is positive.

2. Azo dye test : Phenols are common coupling agents in the preparation of azo dyes. (You will learn about preparation of an azo dye in expedriment 18). This may be used as a test for phenolic group. The test can be performed as follows.

Take 2-3 drops of aniline in about 3 cm^3 of dil. HCl and shake to get a clear solution. Cool the solution in ice bath or you may add a clean, small piece of ice to the test tube. In the meantime dissolve about 200 mg of the phenol in 3-4 cm^3 of 10% NaOH solution. Mix the two solutions. Formation of orange, scarlet or red coloured solution or precipitate indicates the presence of phenolic group.

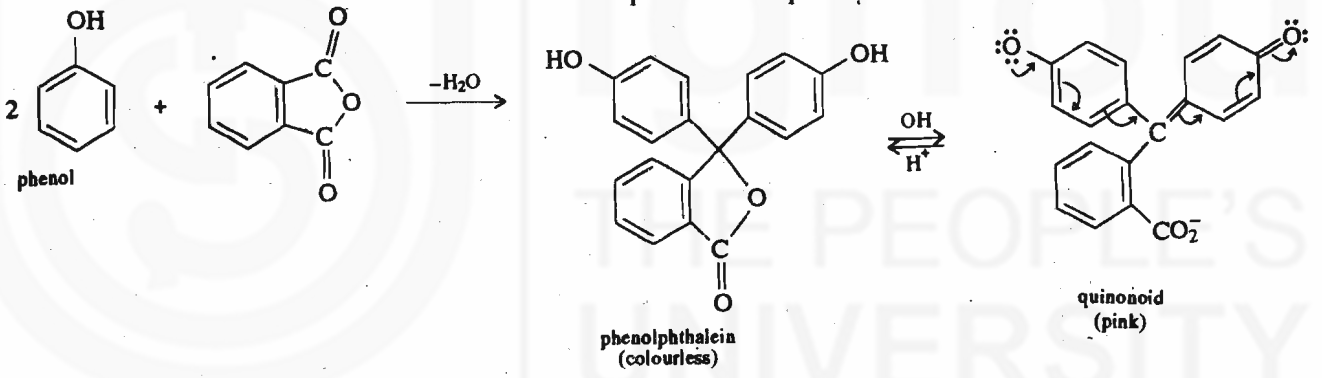
2-naphthol gives scarlet coloured dye



Aniline **Azo dye**

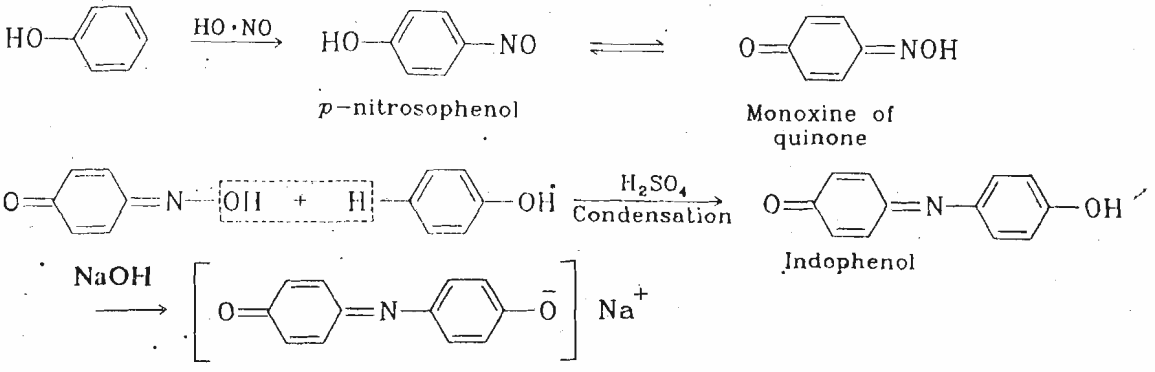
3. Indicator formation or phthalein test : Most phenols condense with phthaleic anhydride to form indicators having blue, red, green or purple colours in alkaline solution. The test may be performed as follows.

Take about 200 mg each of the phenol and phthaleic anhydride in a dry test tube and add 2 drops of concentrated H_2SO_4; gently heat the mixture to fuse it and then let the tube cool down. Add about 1 cm^3 of 10% NaOH solution and stir the mixture. You may need to use a glass rod to break the fused mass. Add more NaOH solution till the solution is alkaline and observe the colour. Red, blue, purple or green colouration indicates the presence of a phenol.



2-naphthol gives faint green colouration

4. Liebermann reaction : . Phenols with free para position show this test. Phenols are made to react with nitrous acid to form *p*-nitroso derivatives. These derivatives react with excess of the phenol to form the indophenols which are acid-base indicators. The reaction for phenol can be represented as follows:



The test is performed as per the following procedure.

Take about 100 mg of the compound in a dry test tube and add 1-2 cm^3 of concentrated H_2SO_4 and a few crystals of sodium nitrite ($NaNO_2$) to it. Shake the test tube and warm a little. Green, blue or purple colour action indicates the

presence of phenolic group. Dilute the contents with about 5-10 cm^3 of water. The colour changes to red (or blue-red). Add concentrated NaOH solution till the solution becomes alkaline. Its colour changes to blue (or green).

Test for Ether group

The third compound of the mixture viz., 1,4-dimethoxybenzene, is a neutral compound and has ether functional group. In general, ethers are quite inert and their identification is quite difficult. Normally they are identified by elimination rather than by direct test i.e., if all the classes/functional groups are absent then the compound may be an ether.

1.6 RESULT

The given mixture has been separated into its constituent i.e., benzene acid, 2-naphthol and 1-4 dimethoxybenzene.

The separated compound have been tested for their functional groups; a carboxylic acid, a phenolic hydroxyl and an ether functional group respectively.

EXPERIMENT 2
SEPARATION OF A MIXTURE OF *p*-TOLUIDINE AND NAPHTHALENE BY SOLVENT EXTRACTION AND IDENTIFICATION OF THEIR FUNCTIONAL GROUPS

Structure

2.1 INTRODUCTION

In the previous experiment you have separated a mixture containing two acidic and one neutral compound. The extracting solutions were aqueous bases. In this experiment you will be seperating a mixture of a basic and a neutral compound. As you will recall from sec. 1.2 of experiment 1 that this will require an aqueous acidic solution. The basic compound after separation is recovered by re extraction.

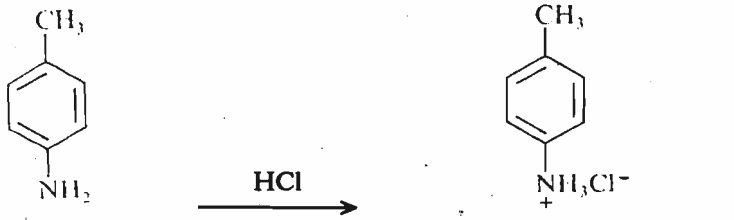
Objectives

After studying and performing this experiment you should be able to :

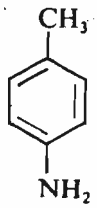
- separate a mixture of a neutral and a basic organic compound by solvent extraction technique,
- identify the functional group of the separated compounds, and
- describe the theory behind the above.

2.2 PRINCIPLE

This mixture contains a basic compound, p-toluidine, and a neutral compound viz., naphthalene. Their separation is quite straight forward. You will recall from above that a basic compound can be extracted by an aqueous solution of an acid. As this mixture contains only one basic compound (as against two acidic compounds in the previous experiment), you don't need to bother about which acid to use for extraction. A strong acid like HCl is definitely a good choice and will afford an effective separation. The hydrochloric acid will convert *p*-toluidine into its hydrochloride salt which on extraction will move into aqueous layer.

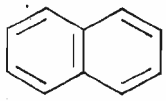


(*p*-toluidine) (*p*-toluidine hydrochloride)

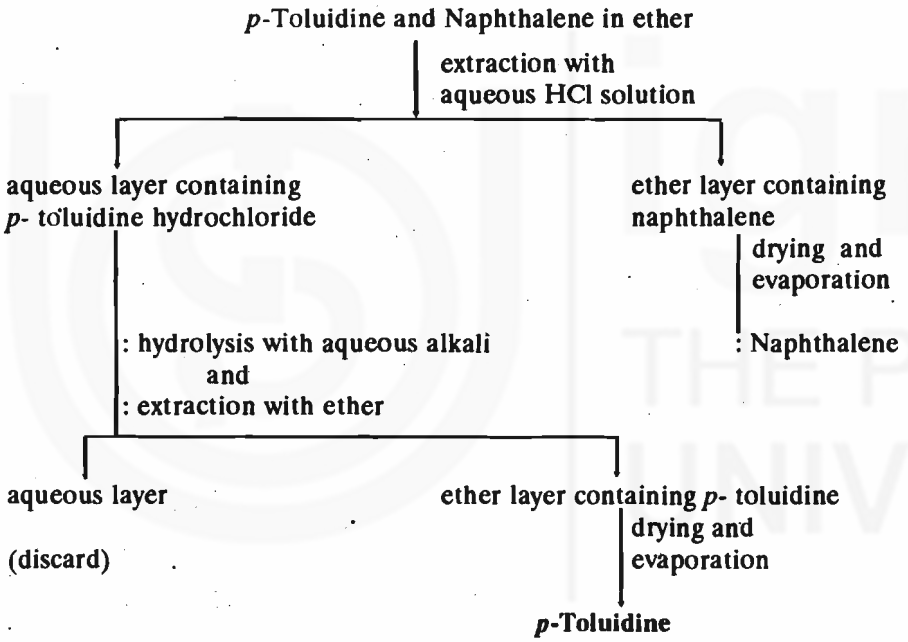


p-Toluidine

Free *p*-toluidine can be recovered by hydrolysing this salt with an aqueous base. The neutral compound, naphthalene, will stay in etheral layer and can be recovered by evaporation of the solution. The separation strategy can be represented as per the following flow chart.



Naphthalene



2.3 REQUIREMENTS

Apparatus		Chemicals
Separatory funnel (100cm^3)	1	Solvent ether
Beakers (100 cm^3)	2	Anhydrous sodium sulphate
Conical flask(50cm^3)	1	Sodium chloride
Ring clamp	1	Sodium hydroxide solution
Funnel (small)	1	Hydrochloric acid
Filter paper		

Solution Provided

1. **1*M* Hydrochloric acid :** This solution is prepared by taking 10 cm^3 conc. HCl and diluting with water up to 100 cm^3.

2. **Sodium chloride saturated solution:** This solution is prepared by dissolving excess NaOH in water.

3. **10 % Aqueous solution of sodium hydroxide :** It an be prepared by dissolving 10 g NaOH in 100 cm^3 of water.

2.4 PROCEDURE

The procedural instructions are given below in sequential order. You are expected to go through the complete procedure and prepare a broad mental outline of the same.

Teflon stopcocks need not be greased

1. Clean the separating funnel first with soap water and then with plenty of water. Grease the stopcock to ensure its smooth movement.

2 Close the stopcock and mount the separatory funnel in the ring support, on an iron stand. If the ring support is not available, you may use a tripod stand for the purpose.

3. Weigh 2 g of the mixture on a rough weighing balance and dissolve it in about 30 cm^3 of solvent ether in a conical flask.

4. Transfer the solution to the separatory funnel after ensuring that the stopcock is closed. Wash the conical flask with a little (5 cm^3) of ether and pour this also into the separatory funnel. This ensures complete transfer of the mixture.

5 Add about 20 cm^3 of 1*M* hydrochloric acid.

If you don't release the pressure then the stopper may be blown off. along with the spilling of the solution.

6. Gently swirl the contents of the funnel to mix them. Release the pressure build up inside the flask. This pressure is due to the evaporation of highly volatile ether. To release the pressure, carefully turn the funnel upside down holding the stopper in place and cautiously opening the stopcock as done in the previous experiment. You will hear the sound of escaping vapours. Close the stopcock again and mix the contents well with repeated release of pressure.

7. Allow the mixture to stand in the funnel (on the ring support) untill the two immiscible layers are separated.

8. Remove the stopper at the top and draw off the lower layer into a 50 cm^3 conical flask labelled 'A'

9. Put additional 5cm^3 of H_2O in the funnel,shake the contents, allow the layers to separate and draw off the lower layer again in flask 'A'.

10. Pour about 15cm^3 of saturated aqueous solution of sodium chloride in the separatory funnel shake vigorously for about a minute and allow the layers to separate. Draw out the lower layer and discard it.

This treatment removes the water which gets dissolved in ether and helps in its drying.

11. Pour the ether layer into a conical flask labelled 'B' containing anhydrous sodium sulphate prepared as described in the previous experiment.

RECOVERY OF SEPARATED COMPOUNDS

By following the above procedure you will obtain two flasks containing the separated compounds.

Flask 'A' : *p*-toluidine as *p*-toluidine hydrochloride in water.

Flask 'B' : naphthalene in ether.

The desired compounds can be obtained as follows

Recovery of p-toluidine : As said above *p*-toluidine can be recovered by hydrolysing the salt with aqueous alkali. Take flask 'A' containing the solution of *p*-toluidine hydrochloride and add dilute NaOH solution dropwise into it with constant shaking. Continue addition till the solution becomes alkaline. This can be checked with the help of a pH paper. When pH of the solution approaches ≈ 10, you may see the separation of *p*-toluidine as a solid. It may so happen that you may get the solid even after adding little more of NaOH solution. This can be because of a low melting point of toluidine (43°C).

If you get the solid filter it and dry the crystals in the folds of filter paper. Report the amount of *p*-toluidine obtained and save the sample for functional group determination. If you do not get a solid but land up with an oily mass or emulsion then proceed as given below.

Transfer the above solution from flask 'A' to a separatory funnel mounted on a ring stand. Put about 20 cm^3 of ether into it and gently swirl it so as to dissolve any *p*-toluidine droplets sticking on the walls. Transfer this ether also to the separatory funnel. Gently swirl the separatory funnel to extract p-toluidine into ether. Keep the funnel for some time so as to allow the layers to separate. Collect the aqueous layer again in the flask 'A' and pour the etheral layer to flask labelled 'C'. Repeat the process with another 20 cm^3 of ether.

Dry the etheral fraction in flask 'C' by putting in some anhydrous sodium sulphate crystals. Decant the ether solution from flask 'C' into another flask. Put about 5 cm^3 of ether into flask 'C' and thoroughly rinse the flask along with drying agent. Wait for a minute or two and mix this ether with the previous lot by completely decanting it. Take care not to transfer the drying agent. Evaporate the ether on a steam bath or preferably distill it.

Recovery of naphthalene : Decant the ether solution from flask 'B' into another flask and proceed exactly as in the above case. Scratch the solid with a neat spatula and weigh it. Report the amount of naphthalene obtained and save the sample for functional group determination.

2.4.1 Test For Functional Groups

Test for primary amines : p-toluidine contains a primary amino group. Generally speaking a large number of tests are available to detect the presence of amines. Some of these tests are applicable to more than one subclasses of amines viz. primary, secondary or tertiary amines. On the other hand there are some tests which are specific to primary, secondary or tertiary amines. Since the compound you have separated is a primary aromatic amine, we are providing tests for primary aromatic amines only.

1. **Diazotisation (or dye) test :** Reaction of nitrous acid with primary aryl amines at low temperature converts them into diazonium salts. These salts are stable at low temperature and couple with sodium salt of 2-naphthol to produce a red coloured solution or precipitate. You will recall that a similar reaction was performed for 2-naphthol in the previous experiment. In a test tube take about 200 mg of the amine (p-toluidine in your case) in about 3-4 cm^3 of dilute hydrochloric acid and cool it in an ice/salt bath. You may put a small clean piece of ice in the test tube and add a few drops of sodium nitrate solution. In a second tube dissolve about 200 mg of 2-naphthol in 3-4 cm^3 of 10% NaOH solution. Mix the two solutions; a brilliant red coloured solution or precipitate (of the dye) indicates the presence of a primary amine.

   To prepare ice/salt bath, take 200 cm^3 beaker and fill it to about two third with crushed ice and sprinkle some common salts add a little water on to it.

2. **Isocyanide test :** Primary amines react with chloroform and alcoholic NaOH to form isocyanides. These are compounds with foul smell and cause nausea. The test is quite delicate and can be used to detect small impurities of primary amines in other amines.

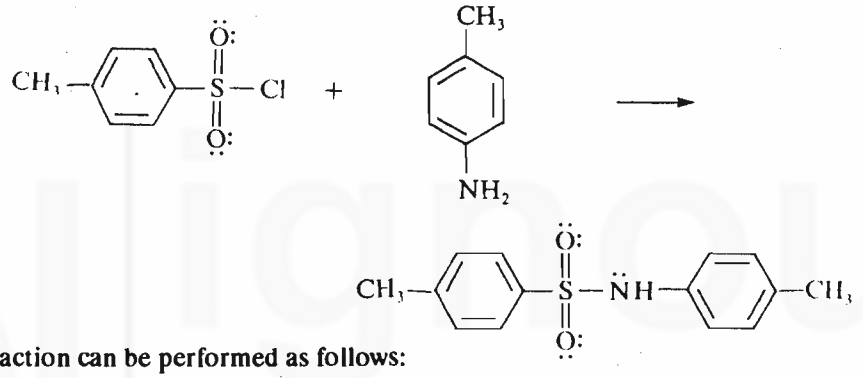
   $C_6H_5NH_2 + CHCl_3 + 3\ NaOH \longrightarrow C_6H_5NC + 3\ NaCl + 3\ H_2O$
   (aniline) (benzene isocyanide)

Take about 200 mg of the aryl amine (*p*-toluidine in your case)and a few drops of $CHCl_3$ in a test tube. Add 2-3 cm^3 of alcoholic NaOH. Thoroughly mix the components, warm gently and note the odour. Nauseatic odour of isocyanide indicates the presence of primary aryl amines.

Add excess of concentrated HCl, after cooling the mixture to destroy the isocyanide. The acid hydrolyses the isocyanide to corresponding hydrochloride of the amine.

The test is negative for secondary and tertiary amines.
Both aliphatic and aryl amines show this test.

3. Hinsberg test: This test in fact is used to differentiate between primary, secondary and tertiary amines and can as well be used to establish the presence of primary aryl amines. In this test the suspected primary aryl amine is made to react with *p*- toluene sulphonyl chloride or benzene sulphonyl chloride. The *p*- toluene sulphonamides or benzene sulphonamides produced are soluble in alkali. The reaction of *p*-toluene sulphonyl chloride with *p*-toluidine can be represented shown below:



The reaction can be performed as follows:

The *p*-toluene sulphonamides of secondary amines do not dissolve in NaOH. The tertiary amines do not form *p*-toluene sulphonamides.

In a test tube take about 100 mg of the amine in 2 cm^3 of alcohol and add about 400 mg toluene sulphonyl chloride. Heat the mixture on a steam bath upto about 60°C and add 4 cm^3 of 20 % NaOH solution. Cork the tube and shake vigorously for about five minutes and keep the mixture for some time with occasional shaking. Dilute the mixture with 2 cm^3 of water and acidify with dilute hydrochloric acid. The *p*-toluene sulphonamide precipitates out. Filter the precipitate and dissolve it in 5% NaOH solution. The dissolution of precipitate confirms the primary nature of aryl amines.

Test for aromatic hydrocarbons : The second compound in the mixture viz., naphthalene is an aromatic hydrocarbon. It does not have any functional group. Generally aromatic hydrocarbons burn with sooty flame and are insoluble in water like ether the primary aromatic hydrocarbon is also established by elemination.

You may try the following colour test for naphthalene.

Add two drops of formalin to 2 cm^3 of concentrated sulphuric acid in a test tube. In a second test tube prepare a solution of about 100 mg of naphthalene in 1 cm^3 of CCl_4. Add two drops of this solution to the formalin solution and observe the colour. Naphthalene gives a dark red colour solution with a black precipitate of resinous substance.

2.5 RESULT

1. The given mixture has been separated into its constituents viz. p-toluidine and naphthalene

2. The separated compounds have been tested for their functional groups.

EXPERIMENT 3
EXTRACTION OF CAFFEINE FROM TEA LEAVES

Structure

3.1 INTRODUCTION

In the previous two experiments you have used the technique of solvent extraction to separate acidic and/or basic compounds from their mixtures. Another important application of solvent extraction is the extraction of important compounds from the materials obtained from natural sources. A large number of medicinally important compounds, scents and oils etc. are obtained by this method. In this experiment you will learn about extraction of a very common and important natural product viz., caffeine from tea leaves.

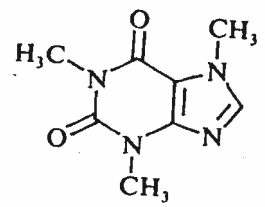
The term 'alkaloid'proposed by W. Missner in 1819, meant 'alkali- like' and was applied to basic nitrogen containing compound of plant origin. These are physiologically active compounds and are normally bitter and have complex structures. Nicotine (active material of tobacco), cocaine (a local anaesthetic), morphine (a narcotic analgesic), peprine (constituent of black pepper) and coniine (the toxic constituent of the notorious poison 'hemlock ' that caused the death of SOCRATES) are some of the examples of alkaloids.

Caffeine (1,3,7 -trimethylxanthine) is probably the most extensively used (knowingly or unknowingly) stimulant. It is an alkaloid and stimulates respiratory, circulatory and central nervous system. It is believed to help in digestion, recovery from depression and treatment of 'gout', a disease of joints and is a well known diuretic i.e., promotes urination. On the negative side it is highly habit forming i.e., leads to addiction. You would have heard of or experienced the addiction to tea and coffee. A tea/coffee addict may feel insomnia (sleeplessness) or headache on not taking tea\ coffee for a long time.

Objectives

After studying and performing this experiment you should be able to.

- extract caffeine from tea leaves
- purify the sample so obtained, and
- outline the importance of caffeine.



Caffeine

Cola drinks contain cola nuts, a source of caffeine.

Tannins are derivatives of gallic acid. tea

3.2 PRINCIPLE

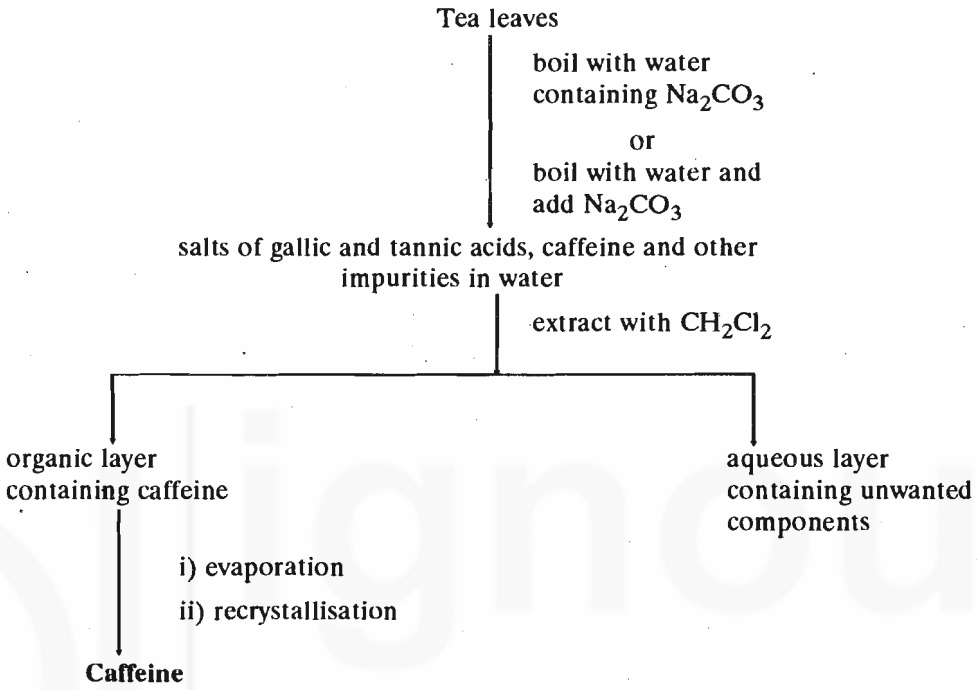
Tea, coffee and cola nuts are the most common and rich sources of caffeine.In the present experiment we are going to use tea leaves as the source of caffeine. Tea leaves contain gallic acid, tannins and a number of other coloured compounds in addition to caffeine.

Caffeine is quite soluble in water, 100 cm^3 of boiling water may dissolve as much as 67.0 g of caffeine. So caffeine can be extracted quite easily into hot water. Yet, the extraction procedure is not that straight forward.It is so because boiling water extracts a number of other species (mentioned above) in addition to the desired

caffeine. We need to separate these unwanted materials. This necessitates a kind of double extraction.

In the process of tea making boiling/soaking of tea leaves in hot water essentially involves extraction of caffeine.

First we boil the tea leaves with water, i.e., extract caffeine and other species into aqueous medium then we convert the acidic impurities into their sodium or calcium salt and reextract caffeine into dichloromethane or chloroform. This procedure may schematically be represented as follows



You may be wondering that why don't we make single extraction with dichloromethane.You are right in thinking so but experiments have shown that in such an attempt the extent of extractions is to the tune of about 20% of that obtained with water extraction. Smelling of tea leaves in water may be the cause of better extracting properties of water.

3.3 REQUIREMENTS

Apparatus		Chemicals
Separatory funnel (500cm^3)	1	Sodium Carbonate
Beakers	2	Dichloromethane
Funnel	1	Acetone
Kjeldhal flask	1	Petroleum ether
(or melting point apparatus)		Anhydrous sodium sulphate
Measuring cylinder (100 cm^3)	1	Tea leaves
Conical flask (500 cm^3)	1	

3.4 PROCEDURE

The procedural instructions have been given below in sequential order. You are expected to go through the complete procedure and prepare a broad mental outline of the same.

Extraction of Caffeine from Tea Leaves

1. Take about 300 cm^3 of water with a measuring cylinder in a 500 cm^3 conical flask and add about 30 g of tea leaves (or 6 tea bags of 5 gm each) and 22.5 g of sodium carbonate to it.

2. Heat the flask till the contents start boiling, reduce the flame and continue heating for another ten minutes, with occasional stirring or swirling of the mixture.

3. Allow the mixture to cool to room temperature (it takes about 5- 10 min) and then decant the extract into another conical flask.

4. Add about 25.0 cm^3 of hot water to the first flask and shake the contents for about 2 min. Decant the washing liquid and mix it with the previous extract.

5. After cooling the extract to room temperature, transfer it to separatory funnel. To this add about 30 cm^3 of dichloromethane and gently swirl the funnel for 2-3 min to extract caffeine.

Make sure that the solution is not hot, otherwise dichloromethane (with very low boiling point), would evaporate off.

6. Release the pressure built up inside the funnel and keep the funnel in the ring support till the two layers separate out.

7. Draw out the lower, dichloromethane, layer into a 250 cm^3 conical flask. **Caution : Do not shake the mixture vigorously.It would lead to the formation of emulsions.Gently swirl or shake the separatory funnel. If you still get an emulsion try to break it with the help of a stirring rod or by adding a small amount of dichloromethane. If this also fails, add about 10 cm^3 of saturated solution of sodium chloride, shake gently and allow to settle. This is called SALTING OUT. Emulsions result from the solubility of CH_2Cl_2 in water brought by certain substances present in tea.**

8. Repeat the extraction twice more and combine all the extracts (include the emlsion, which you could not break, also) and add about 5g of anhydrous sodium sulphate to it. Shake and keep for some time.

9. Decant the dichloromethane solution carefully into a distillation flask and distill off all the dichloromethane till you get a greenish white residue of caffeine.

10. Dissolve this residue in about 5-10 cm^3 of acetone on a water bath and add petroleum ether along the sides of the flask till the solution acquires a cloudy appearance. Allow it to cool to room temperature and keep it in an ice bath for some time.

11. Filter the crystals obtained and wash them with small amount of petroleum ether. Dry and weigh them.

12. Take the melting point of the sample so obtained.

13. Report the yield and the melting point.

3.5 RESULT

1) ..... g of caffeine was obtained from 30 g of tea leaves.

2) The melting point of the caffeine obtained is found to be ..... °C.

EXPERIMENT 4
PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF METAL IONS

Structure

4.1 INTRODUCTION

Experiments 1, 2 and 3 were concerned with solvent extraction methods. Our subsequent experiments 4 to 9 will be concerned with chromatographic separation methods. Here we shall first discuss the basic theory of chromatography with some fundamental concepts and then the principle of liquid chromatography, after that you will be introduced to the actual experiments in which you will perform paper chromatography of metal ions and sugars (Experiments 4 and 5), thin layer chromatography of amino acids (Experiment 6), column chromatography of natural pigments and of inorganic substances (Experiments 7 and 8).

Objectives

After studying and performing this experiments you should be able to:

- define and classify chromatography,
- understand theoretical principle of chromatography,
- calculate the R_f values, and
- Separate and identify cations of analytical group I and II

4.2 THEORY OF CHROMATOGRAPHY

4.2.1 Definition

Chromatography is referred to any of a diverse group of techniques that effect a separation through a distribution of sample between two immiscible phases. One phase is stationary whereas the second is mobile which percolates through the first phase. The stationary phase may be a solid or a liquid while the mobile phase may be a liquid or a gas.

4.2.2 Classification

There are various ways to classify chromatography.

1. On the basis of physical states of mobile phase the chromatography is classified into two board groups.
   - Liquid chromatography in which mobile phase used is in the form of a liquid.
   - Gas chromatography in which mobile phase used is a gas.
2. On the basis of physical states of **stationary phase** and its working principle, chromatography is classified as:
   - Adsorption chromatography in which stationary phase is a solid and works as an adosorbent.
   - Partition chromatography in which stationary phase is a liquid or a liquid supported on an inert solid, and the movement of solute is based on the partition coefficient of the solute into two phases.
   - Ion exchange chromatography in which stationary phase is an ion exchanger and the distribution of solute is based on the ion exchange principle.
   - Gel chromatography in which stationary phase is gel and separation is based on its sieving action.
3. On the basis of the types of column it may be classified as:
   - Column chromatography in which a closed column containing the stationary phase in a cylindrical tube is employed.
   - Sheet chromatography using an open column system in which separations are achieved on sheets of filter paper or thin layers of certain fine solid particles supported on glass or plastic plates.

4.2.3 Principle

Chromatography is essentially a separation process which affects a separation by distributing the sample into two phases. One phase is stationary and second is mobile and flows through the stationary phase. During the process of movement of mobile phase, small differences in adsorption-desorption or partitioning or ion-exchange behaviour of each component of a mixture are multiplied many fold and these parameters distinguish between the different solutes. The ability of chromatography to separate two solutes, depends on the selectivity of the process and the degree to which the system can distinguish between the two solutes. The magnitude of the distribution is determined by the physico chemical nature of the solute and that of the mobile and stationary phases, beside various physical interaction (such as: hydrogen bonding, dipole moment etc.) of the solute with stationary and mobile phases.

Some common steps used in different chromatographic techniques are: application of the sample onto a stationary phase, percolating a mobile phase over the stationary phase, obtaining the separation of components, collection of the different components for qualitative or quantitative purposes. Therefor, the principle of chromatography can be understood by taking a one kind of chromatogaphy. For this

purpose, let us consider a simple example of separation by column chromatography. This can be applied to other methods as well.

Let us suppose that we wish to separate a mixture of two (say coloured) components A and B. A small amount of the sample solution is introduced at the top of the column which is packed with suitable adsorbent (stationary phase). First a narrow band is formed at the top of the column. The developer (mobile phase) is now poured into the column and is allowed to flow through the column. The two important steps are: (i) formation of the initial zone (ii) development of the initial zone and allowing the components to appear as separate zones.

As soon as the solution comes in contact with the stationary phase (column material or adsorbent) and the mobile phase the following reversible reaction occurs:

Solute in the Stationary phase $\rightleftharpoons$ solute in the mobile phase

For which the equilibrium concentrations are related as

$$K' = \frac{Cm}{Cs} \text{ or } K = \frac{Cs}{Cm} \qquad ...(4.1)$$

where, $K' = 1/K$ and K' is equilibrium constant

K is called as the distribution coefficient, Cs is the concentration of the solute in the stationary phase and Cm that in mobile phase.

For two solutes A and B there is competition of A and B (i) for the stationary phase and (ii) for the mobile phase.

If the stationary phase takes B more likely than A, the value of K_A will be less than K_B. Thus when the developer leaves the narrow band it is richer in A than B. The developer flowing downwards now comes in contact with fresh stationary phase and the solutes A and B face the new competition and when the mobile phase leaves this part of the stationary phase, each time it becomes richer and richer in A. Small differences in the interactions of A and B with stationary and mobile phases become exaggerated as the developer proceeds down the column, soon after the well separated bands of A and B are obtained.

If the development further proceeds (elution analysis) the components A and B of the mixture are elute out of the column. Solute A which has smaller K emerges first followed by B which has a larger K. The two fractions may be quantitaively analysed for A and B respectively.

4.2.4 The concept of R_f Value

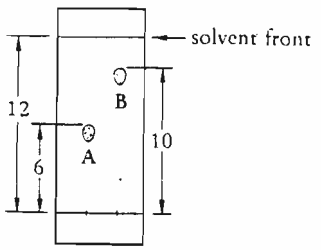
R_f value of a solute is the ratio of the rate of movement of the solute peak to the rate of movement of the eluting solvent.

However, one can not readily detect the position of the solvent front or of the solute on the column chromatography. These are better measured now in terms of retention volume or retention time.

In paper chromatography R_f value is constantly quoted as a characteristic of the solute. It describes the migration of solute relative to that of developer and is given by

$$R_f = \frac{\text{distance moved by the centre of the solute zone}}{\text{distance moved by the solvent front}}$$

With this background, now we will take up experiment based on different types of chromatographic separations in detail.



$$R_f = \frac{\text{Distance to leading edge of spot}}{\text{Distance to solvent front}}$$

$$\text{A } R_f = \frac{6}{12} = 0.50$$

$$\text{B } R_f = \frac{10}{12} = 0.83$$

Measureing R_f values in paper chromatography.

4.3 PAPER CHROMATOGRAPHIC SEPARATION OF METAL IONS

Paper chromatography (PC) is a simple technique to separate complex mixtures of metal ions, amino acids, sugars, dyes and drugs. A very small amount of sample is required for the analysis. PC has become a popular technique for the separation of metal cations. In this experiment, the use of PC will be illustrated to separate a mixture of cations of group I and group II. Next experiment concerned with PC separation of the sugars.

4.3.1 Principle

In paper chromatography of cations, the principles of partition, adsorption and ion exchange may be exploited, out of these the most important is partition the involves the distribution of a solute between a mobile liquid phase and a gel (a kind of water cellulose complex) as the stationary phase. The different components of the sample are distributed across the paper depending on their partition coefficients.

Attempts have been to employ paper chromatography for the systematic qualitative analysis of metal cations. However, it is not possible to separate all the cations simultaneously and therefore, the separation of a group of cations can be handled. Most frequent of these is the preliminary separation into the current analytical groups, each of which is then subjected to a separate chromatographic analysis. In the two parts of this Experiment (4a and 4b) you will learn the use of paper chromatography for the separation of cations of analytical group I and group II, respectively.

4.4 EXPERIMENT 4A: SEPARATION AND IDENTIFICATION OF CATIONS OF ANALYTICAL GROUP I

This experiment of an easy ascending paper chromatographic technique is rapid and requires no special apparatus or reagents. A very simple procedure for this separation has been employed with the use of only distilled water as the developer. However, other developers can also be utilised depending on the availability of the reagents and precautions in their use [e.g. butanol - 1 + pyridine + water (15:1:9)]. In this experiment you will perform the separation of cations of group I.

4.4.1 Requirements

Apparatus		**Chemical**
Boiling tubes or chromatographic jar	5	Potassium chromate
Measuring cylinder	1	Lead nitrate
Pipette	1	Silver nitrate
Spotting capillaries	5	Mercurous nitrate
Small test tubes	5	Nitric acid
Whatman No. 1 Filter paper		

Solution provided

1) **Unknown solution:** It can be prepared by dissolving any one or two nitrate of analytical group I in water.

2) **Detector**: 0.25 *M* aqueous solution of potassium chromate $K_2Cr_2O_4$ is prepared by dissolving 24.25 g K_2CrO_4 in distilled water in 250 cm^3 volumetric flask.

4.4.2 Procedure

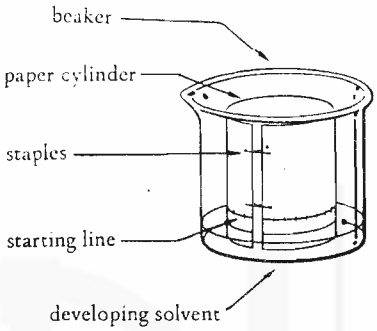
Proceed according to the following steps:

1) **Preparation of solutions**

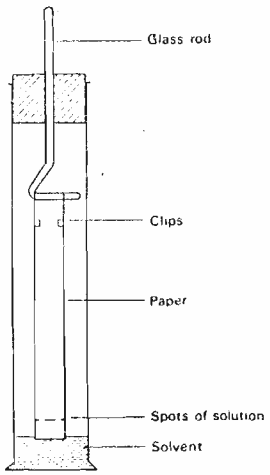
   i) Prepare 1 cm^3 aqueous solutions of (i) lead nitrate (ii) silver nitrate (iii) mercurous nitrate by dissolving about .1g crystals in a small test tube. Add few drops of HNO_3 to prevent hydrolysis. For the preparation of mixture of cations, add few drops of each cation solutions in a test tube.

   ii) Developer: Distilled water.

2. Cut whatman No. 1 filter paper strips of the required size: 15 cm × 2 cm to fit usual boiling tube or 15 cm × 3 cm for chromatographic jar.



An Alternate mathod for developing paper chromatogram by making paper cylinder. Beaker can be covered by aluminum foil.



Apparatus for paper chromatography

3. On each strip draw a line at about 1 cm of the one and put a dot in the centre of line. This and will be the bottom of the strip and development will take place from this end.

Application of the sample on the paper should be on a small area. Larger spots lead to poorer separations.

4. Apply the solutions of Pb^{2+}, Ag^{+} and Hg_2^{2+} separately on 3 strips with the help of a fine capillary. Use a fresh capillary for each solution. The teacher is supposed to demonstrate the technique of application of the solution.

Please indicate the name of test sample on the top of the paper strips.

5. On the 4th paper strip apply the mixture of the three cations.

6. Apply the unknown solution (e.g. containing any one or two on the 5th paper strip.

The paper in the boiling tube or in the jar should be vertically changed and it should not touch the sides of the tube.

7. Place 5 dry boiling tubes vertically in a stand.

8. Add distilled water, with the help of a pipette, to each of the boiling tubes so that the height of the developer (distilled water) in each of the boiling tubes is less than 1 cm. The sides of the oiling tube must be dry as far as possible.

The spot of the solute (at the point of application) should always be above the level of the developer otherwise the solute will mix with the developer and error will be resulted.

9. Suspend the spotted and dried paper strips in the respective boiling tubes containing distilled water with the upper end pinned to the cark and the lower end touching the developer. Care is taken to see that this is done gently and the strip is vertical. The spot should always be above the developer level.

Ensure that the spot gets dried before placing the paper in the boiling tube.

10. Allow the developer to rise along the paper and wait till the developer (solvent front) reaches near the upper and of the paper.

Do not allow the paper to come in contact with impurities.

11. Remove the paper from the boiling tube and mark the solvent front with the help of a pencil.

12. Get the dried to evaporate the developer.

13. Take potassium chromate solution in a pettri dish (or a watch glass) and dip the dried paper in the detector.

14. Encircle the coloured zones with pencil and mark the centre of the zone.

15. Calculate the R_f values.

16. Compare the R_f values of individual cations with that of their R_f values in known mixture and in unknown.
17. Identify the cations present in the unknown on the basis of R_f values.

4.4.3 Observations and Calculations

Observe the coloured spots of different cations. Pb^{2+} will appear as yellow; Ag^+ as orange-red and Hg_2^{2+} as orange zones. Measure the distance of the centre of each solute zone from the point of application call this distance ds. Measure the distance between the solvent front and the starting line and call this distance as dm.

Calculate the R_f value of each solute by the relation:

$$R_f = \frac{\text{Distance travelled by the centre of solute zone}}{\text{Distance tranvelled by the solvent front}} = \frac{ds}{dm}$$

Record your data in the following way:

Observation Table
PC of Metal ions of group I

Sample	ds	dm	R_f= ds/dm Remark
Ag^+			
Hg_2^{2+}			
Pb^{2+}			
Mixture			
Unknown			
1.			R_f resembles with .....
2.			R_f resembles with .....

4.4.4 Result and Discussion

Metal ions present in the unknown sample are:

1. .....

2. .....

The metal ions of analytical group I move along the paper in distilled water at different rates. On dipping in detector. K_2CrO_4 solution the coloured precipitates of the chromates of lead, mercurous and silver appear as yellow, orange and orange-red zones on the paper. Sometimes, when the coloured spot of Hg_2^{2+} is not intense, the paper is exposed to ammonia vapours: the mercurous compound gives black spot.

Lead migrates with the fastest rate and appears at the upper most area on the paper. Mercurous follows the lead. Silver migrates with the slowest rate and appears below the mercury zone.

4.5 EXPERIMENT 4B: SEPARATION AND IDENTIFICATION OF CATION OF ANALYTICAL GROUP II

In this experiment you will perform the separation of Bismuth (III), copper (II), cadmium (II), lead (II) and mercury (II) using paper chromatography. This separation is achieved by 1- butanol saturated with 3*M* HCl as developer. However, another simple developer containing: ethanol + water + 1 *M* HCl (18:1:1) can also be used to separate Pb^{2+}, Cu^{2+}, Cd^{2+} (excluding Hg^{2+} from Group II).

4.5.1 Requirements

Apparatus		Chemicals
Boiling tubes	5	1-Butanol
Measuring cylinder	1	3*M* HCl
Pipette	1	bismuth chloride
Cupric chloride		Lead chloride
Spotting capillaries	7	Mercuric chloride'
Small test tubes	7	

Pettridish

Whatman No. 1. filter paper sheets.

Solution provided

1. **Unknown solution:** It can be prepared by dissolving any one or two chlorides of analytical group II in water.
2. **Preparation of Detector 1:** H_2S water. It is prepared by passing H_2S gas in water and add a few drops of ammonia to it. Keep it in a covered container.
3. **Preparation of Detector 2:** Dithizone. Prepare a conc. solution of dithizone in chloroform or carbon tetrachloride.

4.5.2 Procedure

Proceed according to the following steps.

1. i) **Preparation of Solution:**

   Sample Preparation : prepare 1cm^3 concentrated aqueous solutions of (i) bismuth chloride (ii) cadmium chloride (iii) cupric chloride (iv) lead chloride and (v) mercuric chloride. Add 1-2 drops of hydrochloric acid to prevent hydrolysis. For the preparation of the mixture of there cations, add few drops of each cation solutions a test tube.

   ii) Preparation of Developer: Developer (1-butanol saturated with 3 *M* HCl): Prepare this developer by taking equal volumes of 1-butanol and 3 *M* hydrochloric acid in a separatory funnel. Shake well and allow to stand to separate the layers clearly. Reject the lower aqueous layer and use the upper (organic) layer as the developer.

2. Cut whatman No. 1 filter strips of about 15×2 cm to be placed in usual boiling tubes (or chromatographic jars).
3. On each strip draw a line with pencil at a bout 1 cm of one end and mark a point in the centre of line. This point is the point of application of the solute/sample solution.
4. Apply the test solution to the point of application with the help of of a fine capillary. Apply bismuth. Cadmium, copper, lead and mercury solutions separately on 5 strips. Use a fresh capillary for each solution.
5. Apply the mixture solution and unknown solution separately on other strips. Application of solution can be repeated twice or thrice if the solutions are dilute.
6. Take the clean and dry boiling tube and place (10-15 cm^3 of) the developer in each of these boiling tube.

7. Suspend the spotted and dried paper strips in the respective boiling tubes containing distilled water with the upper end pinned to the cork and the lower and touching the developer. Care is taken to see that this is done gently and the strip is vertical. The spot should always be above the developer level.
8. Allow the developer to rise along the paper and wait till the developer (solvent) reaches near the upper end of the paper.
9. Remove the paper strip and mark the solvent front with the help of a pencil.
10. Leave the paper for some time to get it dried.
11. Hold the paper in an atmosphere of H_2S gas until the zones of metallic sulphides are seen. An alternative way is to use H_2S water in which the paper is dipped to locate the zones. Another alternative method is use dithizone for detecting the zones of metal ions. Spray the paper (or dip in) with a concentrated solution of dithizone in chloroform.
12. Encircle the coloured zones and mark the centre of each zone. Calculate the R_f values of individual cations with that of their R_f values in mixture to identify the cations in the mixture and unkown sample solution.

4.5.3 Observations and Calculations

Observe the colour of the spots of various cations of group II. With H_2S bismuth (III) will appear as dark brown: cadmium (II) as yellow: copper (II) as chocolate brown: lead (II) as black and mercury (II) as black zones.

Measure the distance travelled by the centre of the solute zone (ds) and the distance travelled by the solvent front (dm) on the paper chromatogram.

Calculate the R_f values of each solute by the relation: R_f = ds/dm.

Record your data in the following way:

Observation Table
PC Separation of Pb^{2+}, Cu^{2+}, Cd^{2+}, Bi^{3+}, and Hg^{2+}

Sample cation	ds	dm	R_f = ds/dm Remark
Hg^{2+}			
Cd^{2+}			
Bi^{3+}			
Pb^{2+}			
Cu^{2+}			
Mixture			
Unknown			R_f resembles with .....
1.			R_f resemble with .....
2.			

Result and Discussion

Metal ions present in the unknown sample are:

1. .....

2. .....

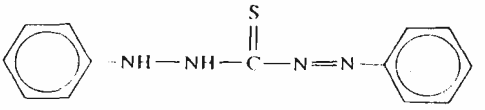
The rate of migration of group II metal ions inn butanol saturated with 3*M* HCl appears in the order $Hg^{2+} < Cd^{2+} < Bi^{3+} < Pb^{2+} < Cu^{2+}$

When H_2S is used as detector the coloured zones of metallic sulphides are seen: HgS black, CdS yellow, Bi_2S_3 dark brown, PbS black and CuS Chocolate brown.

When dithizone is used as detector, coloured complexes of metal ions are formed with dithizone. Dithizone is the commercial name of diphenyl-1-thiocarbazone (see formula in below).

Metal ion + Dithizone ⟶ coloured complex

Colours of the complexes of metal ions are: mercury-pink, cadmium- purple, bismuth-purple and copper brown. Lead is not clearly detected for which rhodizonic acid may be used.



Diphenylthiocarbazone
(dithizone)

EXPERIMENT 5
PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SUGARS

Structure

5.1 INTRODUCTION

In the last experiment you have separated metal ions by paper chromatography.

In this experiment you will again to use paper chromatography for the separation of sugars.

The term sugar applies to mono-, and poly-saccharides, which are all soluble in water and thereby distinguishable from polysaccharides. Many natural sugars are sweet. however, the sweetness varies greatly with stereochemical configuration.

PC of sugars has proved to be of great significance for both qualitative and quantitative separations. On large scale it has proved to be of immense value in the sugar industries, in the analysis of fruit juice and in a number of other fields.

Various developers may be used for the particular PC separations. The developers which are used at present may be modified in order to achieve more satisfactory separations of certain sugar mixtures. However, the methods to separate the optical isomers i.e. to separate corresponding d-and 1-sugars have not yet been developed.

Objectives

After studying and performing this experiment you should be able to:

- explain the principle of paper chromatography,
- separate and identify sugars by paper chromatography

5.2 PRINCIPLE

Distribution of solute (sugar) between the stationary and mobile phases, that is the partition process is the major factor in the PC separation of sugars. Their partition coefficients are substantially in favour of the aqueous phase. Therefore, with non-aqueous developers, sugars appear on the paper choromatogram with low R_f values, whereas with developer containing larger aqueous ratio, the R_f values of sugars are much higher. This is because a sugar molecule containing larger number of hydroxyl groups which is readily soluble in water and makes the partition coefficient in favour of the aqueous phase. Further, the R_f values of sugars are affected by their structural formulae, their molecular mass, the number of-OH groups, and presence of other kinds of groups such as aldehydes or ketones etc.

In this experiment you will learn the PC separation of simple sugars

5.3 REQUIREMENTS

Apparatus		Chemical
Boiling tubes	5	sugars
Measuring cylinder (100 cm^3)	1	Detector (list in given below)
Spotting Capillaries		1-Butanol
Spraying-bottle	5	Acetic acid
Whatman No.1 filter paper sheets'		

Solution Provided :

1. **Unkown sugar sample solution :** It can be prepared by dissolving any one or two sugars in water.
2. **Detector:** Any one of the following detectors may be prepared.

   Detector-1: Ammoniacal silver nitrate: Take 5 cm^3 of saturated aqueous solution of silver nitrate add 50 cm^3 of acetone, finally add ammonia solution to make the solution clear and basic in nature.

   Detector-2: Aniline hydrogen phthalate: Dissolve 1 cm^3 of aniline and 1.66 g of phthalic acid in 100 cm^3 of 1-butanol saturated with water.

   Detector-3: *p*-Anisidine hydrochloride in 100 cm^3 of 1-butanol.

   Detector-4: (For non-reducing sugars): Prepare a solution by mixing 0.25*M* sodium borate + phenol red + methanol in (1:2:7) proportion.

   Developer: In a separatory funnel take 1-butanol + acetic acid+water in the proportion (4:1:5) and shake gently. Allow the layers to settle. Remove the lower aqueous layer and take the upper organic phase (layer) as the developer for sugars.

5.4 PROCEDURE

Proceed according to the following steps.

1. **Preparation of Solution**

   i) Sample solutions : Prepare the aqueous solution of any three of the following by dissolving 0.2-0.5 g of each sugar in 5 cm^3 of water in a small test tube. The sugars are:

   D-gulcose, D-fructose, D-xylose, L-rhamnose, D-galactose, Lactose, maltose, sucrose, D-mannose.

   ii) Preparation of mixture solution of sugars: Add few drops of each sample sugar solution in a dry test tube.

For analysis of sugars from plant or fruit juices, first the material is stored, then the sugars are extracted by grinding the materials in presence of a suitable solvent and finally the non- carbohydrate-material is removed from the extract. The extract can be applied directly or after concentration.

The extract from the fruits contain many other substances along with sugars, for example proteins and organic acids are frequently present in reasonable concentrations to affect the quality of choromatogram.

During the storage of biological material there is always danger of changes in the composition by fermentation of sugars due to the presence of micro-organisms (enzymes).

Unknown Sugar solution: It can be prepared by dissolving any one or two sugar in water.

2. Cut the chromatographic paper strips of the required size.
3. On each strip draw a line with pencil at about 1 cm from one end and put a mark at the centre of the line. The sample is to be applied at this mark. Write the name of a particular sugar on the upper side of the paper with pencil.
4. Apply the respective sugar solution to the point of application separately on the marked strips. Use a fresh capillary for each solution.
5. Apply the mixture solution and the unknown solutions separately on other strips.
6. Dry the spots by allowing the solvent to evaporate.
7. Take the clean and dry boiling tubes and place 10-15 ml of the developer in each of these tube.
8. Suspend the spotted and dried paper strips in the respective boiling tubes containing distilled water with upper end pinned to the cork and the lower end touching the developer. Care should be taken to see that this is done gently and the strip is vertical. The spot should always be above the developer level.
9. Allow the developer to rise along the paper and wait till the developer (solvent front) reaches near the upper end of the paper.
10. Remove the paper strip from the boiling tube and mark the solvent front with the help of a pencil.
11. Dry the strip until the acetic acid odour from the strip is no more present.
12. Treat the strip with a detector by a spraying bottle.
13. Heat the strip at 105 °C in an oven until the coloured zones of sugars are seen.
14. Encircle the coloured zones and mark the centre of each zone.
15. Calculate the R_f values and compare the R_f values of individual sugars with that of their R_f values in mixtures to identify the sugars present in the mixture/sample solution.

5.5 OBSERVATION AND CALCULATIONS

Observe the colour of the spots of various sugars. The colour depends on the detector used. Measure the distance travelled by the centre of the solute zone (ds) and the distance travelled by the solvent front (dm) on the paper choromatogram.

Calculate the R_f values of each sugar by the relation R_F = ds/dm.

Record your data in the following way:

Observation Table

PC Separation of Sugars of 1-Butanol, Acetic Acid and Water (4:1:5)

Sugar	ds	dm	R_f=ds/dm Remark
Lactose			
D-glucose			
D-fructose			
L-rhamnose			
Mixture			
Unknwon			R_f resemble with .....
1.			R_f resemble with .....
2.			

5.6 RESULT AND DISCUSSION

Sugars present in the unknown sample are:

1.

2.

R_f values of sugars in this developer are low as in the upper organic layer the water content is very low. The colour of the sugar zones on the choromatogram depends on the detector used. For example, using detector-1, reduction of ammoniacal silver nitrate results into metallic silver. Therefore, the reducing sugars give rise to brown-black spots after heating to 100 °C.

A popular spraying reagent is detector-2, aniline hydrogen phthalate. The colour development depends on the following mechanism. Heating of sugar with an acid produces furfuraldehyde which can be condensed with an aromatic amine or phenol to give coloured compounds. Sugars which react can appear as red or brown spots. It is sensitive and popular spraying reagent for aldopentoses, aldohexoses, methylpentoses, reducing disaccharides and some others.

EXPERIMENT 6
THIN LAYER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF AMINO ACIDS

Structure

6.1 INTRODUCTION

In the last two experiment you have studied about the paper chromatography. In this part you will learn how to perform thin layer chromatography for the separation of amino acids.

Thin layer chromatography (TLC) is an efficient method of separating complex mixtures. It is a sensitive, fast, simple and inexpensive analytical technique in carrying out small scale experiments.

One of the important applications of TLC is the separation of amino acids. Amino acids contain both the amino groups as well the carboxylic groups. The most important are the $\alpha-$ amino acids as these are the units from which proteins are made.

In this experiments you will learn the movement of some simple amino acids on silica gel coated plates. TLC of amino acids is based on their distribution between a finely divided powder of an adsorbent and an organic mobile phase.

Objective

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

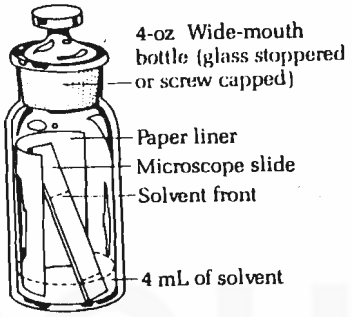
- explain the basic principle of TLC, and
- separate amino acids by TLC

6.2 PRINCIPLE

TLC is similar to PC in that the sample is spotted near one end of a plate of glass or plastic coated with a thin layer of an adsorbent. The TLC plate is place in a covered jar containing a shallow layer of developer. The developer rises up by capillary action and the solute is distributed between the stationary (absorbent) phase and the mobile phase. A solute which is more strongly adsorbed onto the stationary phase, will spend less time in the mobile phase, and hence it will migrate more slowly up the TLC plate. The sample is subsequently separated by development (elution). Treatment with a detector forms the coloured zones of the solutes. The components of a mixture are identified by the calculation and comparison of R_f values.

6.3 Requirements

Apparatus		Chemicals
TLC jar		Propanol-1
(An alternative is a beaker covered		Conc. Ammonia solution
by a watch glass or aluminum foil)		Any three amino acid from the following:
Spotting capillaries		L-alanine
Measuring cylinder100 cm^3		L-Leucine
TLC plates (Either arranged from a	5	L-Lysine
supplier or prepared by the teacher)		L-Aspartic acid
Spraying bottle	1	Methionine

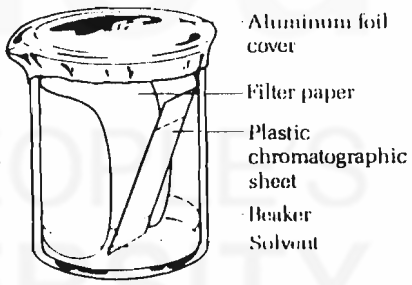


Apparatus for TLC chromatography

Preparation of TLC Plates

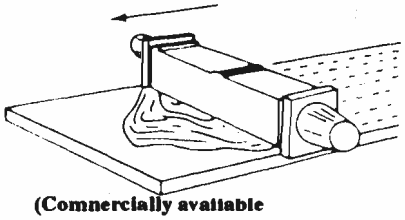
TLC plates can be prepared by one of the following methods:

A. Dipping : Combine 33cm^3 of methanol and 67 cm^3 of chloroform in a 125 cm^3 screw-cap jar, stir in 35g of Silica Gel G, and shake the capped jar vigorously for about a minute, Stack two clean microscope slides back-to-back, holding them together at the top. Without delay dip them into the slurry for about 2 seconds, Touch the bottom of the stacked slides to the jar to drain off the excess slurry, let them air dry a minute or so to evaporate the solvent, separate them and wipe the excess adsorbent off the edges with a tissue paper. Activate the slides by heating them in oven at 110°C for 15 minutes, or by placing them in a covered beaker heated to that temperature.



An alternate method for developing TLC plate

B. Spreading : Take a clean glass plate Mix about 10 g of Silica Gel G with 20 cm^3 of water (stirring and shaking well to get out any lumps) and pour on the glass plate. Spread it out with the help of TLC applicator. Let the plate air dry for ten minutsand put the plate at 110°C in a oven for at least 30 minutes to activate the adsorbent.



(Comnercially available applicator)

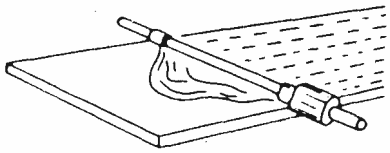
Solutions provided

1. **Sample Solution:** Provide solution of any three amino acid as above. Make one of amino acid solution as unknown sample.

   Their solution can be prepared by dissolving 15 mg of each amino acid separately in 1 cm^3 of distilled water. Warm if a particular amino acid is not soluble in cold.

2. **Detector:**

   Ninhydrin Reagent (0.2%): Take 100 cm^3 of 1-butanol and 100 cm^3 of water in a separatory funnel. Shake gently and allow it to from the layers. Remove the lower aqueous layer. Transfer the upper organic layer to a spraying bottle and to this add 0.2 g of ninhydrin, shake well and use as the detector for amino acids.



(A lab made applicator)

6.4 PROCEDURE

Proceed according to the following steps:

1. Preparation of Developer: Prepare the developer by mixing 1- propanol and concentrated ammonia in the proportion of 7:3 respectively by measuring the required volumes with the help of a measuring cylinder.
2. Take 4 silica gel coated TLC plates from your teacher.
3. Dry the plates in an oven for 30 minutes at 100°C, so that they have been activated for adsorption chromatography.
4. Take a plate and make a light pencil line across it, 1 cm above the bottom of the plate and put a short mark at the line centre where a known or unknown amino acids will be spotted.

Remove a thin strip of the layer from the edge of the TLC plate by means of a thumb nail or a spatula.

5. Label the plate at the top end to indicate known or unknown amino acids.
6. Hold the plate in the left hand cautiously, so that the fingers do not touch the adsorbent layer. Take a capillary and place in the solution of amino acid to be spotted, let the solution rise into the capillary, take out the capillary from the solution and gently touch the capillary to the layer side of the TLC plate at the marked centre. Allow to flow the solution on the plate for a short duration so that a spot of the solution is formed but not larger than 2 mm in diameter.

While marking the plates with pencil, do not press so hard on the pencil that you remove the silica gel.

Due to the limited solubility of various amino acids, great care must be taken in the preparation of the sample before applying.

(**Note:** The teacher is supposed to demonstrate this technique).

7. Allow the spot to dry. You can blow in order to aid evaporation. Apply more solution at the same place (if required). The aim is to apply a small but visible and built up spot.

The spot applied should be kept as small as possible. Application of too much of the solute should be avoided, as this will result in an elongated zone and will affect the correct calculation of R_f values.

8. Apply the unknown solution on a separate TLC plate in a similar manner.
9. After spotting all the known and unknown solutions, insert the plates into the developing jars (one plate in each jar).
10. Pour the mobile phase into the chamber, with the help of a pipette till the developer level reaches nearly at 1 cm height of the lower edge of the adsorbent layer on the plate (Remember that the spot should be above this level).
11. Cover the jar and allow the devloper to ascend along the plate. The position of the solvent front can be seen visually as the damp portion of the plate appears darker than the dry portion.
12. When the developer ascends to a required height on the plate, remove the plate from the developing chamber, mark the solvent front and dry the plate at 100°C for about 10 minutes.
13. After the plates have been dried, spray the detector on the plates with the help of a spraying bottle. The detector is 0.2% ninhydrin solution in butanol saturated with water.
14. Heat the plates at 110°C, either in an oven or on a hot plate, for 5-10 minutes, till the zones of amino acids appear as coloured spots on the plates.
15. Mark the periphery of the coloured spots and their centres.
16. Measure the distance of each spot-centre from the starting line and also the distance by which the solvent front, has moved. Calculate the R_f values.
17. From the comparison of R_f values of known and unknown samples you can determine which amino acids are present in your unknown.

6.5 OBSERVATIONS AND CALCULATIONS

Observe the colour of the spots of various amino acids on TLC plates. Measure the distance to which the centre of the amino acid has moved from the original (ds), and the distance which the solvent front has moved from the point of application (dm) on the chromatoplate.

Calculate the R_f values by the relation

R_f=ds/dm, for each known amino acid and for the unknown. Present the data in a table below.

Observation Table
Separation of amino acids by TLC

Amino Acid	ds	dm	R_f=ds/dm Remark
Leucine			
Lysine			
Alanine			
Methonine			
(known)			
Unknown			R_f resemble with .....
1.			R_f resemble with .....
2.			

6.6 RESULT AND DISCUSSION

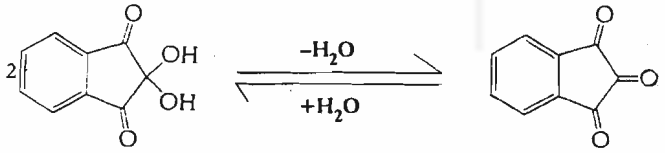
The unknown sample contains:

1.

2.

The mobile phase rises up along the plate by capillary action, rapidly at first, and then more slowly as the solvent front rises. The movement of an amino acid along the TLC plate depends on its adsorptivity, on the adsorbent layer, the solubility in the mobile phase and a number of other factors. Therefore, the different amino acids move along the plate at different rates and may have different R_f values. The significant differences in R_f values of certain amino acids results into a clean separation.

The most widely used reagent for detecting amino acids is ninhydrin. Ninhydrine is the 2-hydrate of indane-1, 2, 3 trione (or triketohydrindene hydrate) with the following formula.



Hydrate form of Ninhydrin

Unhydrated form of Ninhydrin

It reacts with amino acids to yield highly coloured products.

Amino acid + Ninhydrin $\xrightarrow{\text{heat}}$ coloured product.

The formation of visible colour with ninhydrin has limits of detection that may vary from 0.01-0.5 μg depending on the particular amino acids.

EXPERIMENT 7
COLUMN CHROMATOGRAPHIC SEPARATION OF PIGMENTS FROM GREEN LEAVES.

Structure

7.1 INTRODUCTION

In the last three experiment you have studied about paper and thin layer chromatography. In this experiment you will learn how to separate the pigment from green leaves through column chromatography.

Column chromatography is a technique which can be applied to the separation of many complex mixtures. The sample solution is applied to the top of the column. The mobile phase flows down through the column filled with the stationary phase material.

The green leaves of plants contain a number of pigments viz: chlorophyll-a, chlorophyll-b, xanthophylls and carotenes. You will learn the separation of these pigments by column chromatography in this experiment.

Objective:

After studying and performing this experiment you should be able to:

- explain the basic principle of column chromatography,
- prepare the extract (of leaves) for the experiment,
- prepare the calcium carbonate column, and
- separate green pigment (of leaves) by column chromatography.

7.2 PRINCIPLE

The success of a separation by column chromatography depends on the choice of the stationary and mobile phases. The stationary phase material is filled in a column. Any of the three possible mechanisms: partition, adsorption or ion exchange can be employed by the use of a particular type of the stationary phase inside the column. For example, for the separation based on adsorption an adsorbent is packed in the column.

The choice of the mobile phase depends on the nature of the substance and how strongly it is adsorbed. In a number of cases such as alumina and silica gel as the adsorbent, the mobile phase is generally a non-polar solvent such as petrol and benzene because polar groups such as hydroxyl-(OH) group in water and in ethanol would cause desorption. Eluents containing two or more solvents may be used for

better results. In such cases the polarity is increased by adding a polar solvent with a non-polar one.

7.3 REQUIREMENTS

Apparatus		Chemicals
Chromatography column	1	Calcium carbonate
Glass wool		Anhydrous Sodium
Cotton wool		Sulphate anhydrous
Beaker (100 cm^3)	2	Benzene
Conical flask (250 cm^3)	1	Petroleum ether
Mortar	1	Ethyl alcohol
China dish	1	
Separatory funnel	1	
Graduated cylinder (100 cm^3)	1	
Wash bottle	1	

7.4 PROCEDURE

Proceed according to the following steps

1) **Preparation of the Extract:** Take 5-10 g of fresh grass (or leaves of a green plant), cut it up into fine pieces in a mortar, grind for about 30 seconds, add 10 cm^3 of ethyl alcohol and 20 cm^3 of petroleum ether, grind again. Decant the liquid into a separatory funnel after filtering through glass wool placed in an ordinary funnel. Add 10 cm^3 alcohol and 20 cm^3 petroleum ether again to the mortar containing grass, grind and transfer the liquid after decantation to the separatory funnel containing the first fraction. Shake gently. A light green emulsion may form, if shaken vigorously. Allow to settle the layers. The bottom layer is water-ethanol layer and the upper layer of petroleum-ether contains grass extract. Remove the bottom layer and wash the petroleum layer with water for 3 or 4 times until the layer is clear. Remove the aqueous layer. The extract is now free from alcohol but contains water in very small amount.

   Transfer the upper layer containing the extract to a dry conical flask. To this, add anhydrous sodium sulphate (dried by heating in an oven/hot plate before use), shake the flask and leave it over for about 15 minutes to remove any water present with the extract. Transfer the extract to a clean and dry test tube, cover it and take it for chromatography.

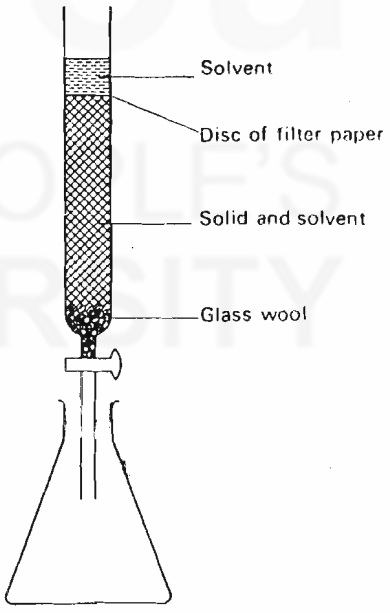
2) **Preparation of column:** Take a glass column or a burette of about 20 cm in length and 7-8 mm diameter tube. Place a small wad of cotton wool as the column support. Pack the column with anhydrous calcium carbonate (dried by heating in a china dish over a burner), tap it regularly with a glass rod. Add the adsorbent in small portions and gently press down until a column of 8-10 cm has been uniformly packed. Place a small wad of cotton wool at the top of the calcium carbonate column and use it for chromatography.

3. Take the uniformly packed column containing calcium carbonate and fix it in a stand vertically.

4. Take 1-2 cm^3 of dried extract of leaves, drip into the column in the form of a thin layer of solution, allow to run evenly into the adsorbent until a green zone 3-4 mm deep is formed at the top of the column. This is known as the loading of the sample.

5. Add the developer (benzene) to the column and allow the developer through the column packing till separate bands are observed.

6. Note the colour of different bands and their order.



Chsomatographic column

The physical state of the column packing material should be such that it allows uniform packing of the column and a free flow of the solvent through it.

The extract from green leaves should be completely free from water since the presence of a polar substance can alter the course of development.

7. If extra time is available, continue the passage of developer and collect the different coloured substances in fractions, noting the volume eluted by a measuring cylinder.

7.5 RESULT AND DISCUSSION

The bands observed on the column are of different colours. The uppermost thin yellowish green zone is chlorophyll-b, below this the bluish green zone of chlorphyll-a, next orange-yellow zone contains xanthopylls and the lowest orange zone contains carotenes. The carotenes are least adsorbed by the adsorbent and can be easily washed out of the column.

Three main interactions are to be considered in column chromatography: the activity of the adsorbent, the polar behaviour of the substance and the polarity of the eluting solvent.

This experiment is based on the results of the inventor of the technique of chromatography, M. Tswett, who applied the technique to separate various plant pigments using calcium carbonate as the stationary phase packed in a column.

EXPERIMENT 8
COLUMN CHROMATOGRAPHIC SEPARATION AND ESTIMATION OF INORGANIC SUBSTANCES:

8.1 INTRODUCTION

In the last experiment you have leart how to separate pigment by column chromatography. In this experiment we will use column Chromatography for the separation of inorganic substance.

Column chromatography with an adsorbent in the column can be used to separate inorganic ions analogous to separate organic compounds. An aqueous solution of salts is introduced to a column of an adsorbent and the column is eluted with water, or dilute acid, or any other suitable eluent. The separated substances (ions) can be eluted out of the column and determined by suitable methods.

In this experiment you have to separate a given mixture containing potassium permanganate and potassium dichromate on a column of alumina. The two components are eluted out of the column and their amounts are then determined titrimetrically.

Objective

After studying and performing this experiment you should be able to:

- prepare a column,
- separate $KMnO_4$ and $K_2Cr_2O_7$ by column chromatography,
- determine the strength of $KMnO_4$,
- determine the amount of $K_2Cr_2O_7$ in the sample.

8.2 REQUIREMENTS

Apparatus		Chemicals
Chromatography column	1	Alumina
Cotton wool/glass wool	1	0.5 *M* HNO_3
Beaker	2	1*M* H_2SO_4
Conical flask	1	0.05 *M* $KMnO_4$

Burette (50 cm^3)	1	0.05 *M* $K_2Cr_2O_7$
Pipette (10 cm^3)	1	0.05 *M* Ferrous ammonium sulphate

Solutions provided

1. 0.5 *M* Nitric acid solution; Prepare 0.5M HNO_3 solution O_3 solution from a stock HNO_3 solution of known specific gravity and percentage.
2. 1*M* sulphuric acid solution: Prepare 1*M* solution from a stock H_2SO_4 solution of known specific gravity and percentage.

   Remember:- never add water to the acid; always acid is added to water for dilution.
3. Prepare by weighing 0.05 *M* $KMnO_4$ and 0.05*M* $K_2Cr_2O_7$ solution in water.
4. Prepare 0.05 M ferrous ammonium sulphate solution by weighing the required amount and transferring to the standard flask containing some dil H_2SO_4 solution to check the hydrolysis, make it to the mark water.

8.5 PROCEDURE

1. **Preparation of column:** Take a glass column or a burette of about 20 cm long and 1-2 cm in diameter. Place some cotton wool into the bottom of the column. Pour nearly 10 cm^3 of hot water and remove air from the wool. Allow to flow some water, opening pinch clamp. Take about 20 g of alumina in a beaker containing 100 cm^3 of water. Heat the beaker to boiling of water to remove the dissolved air. Now transfer a small quantity of alumina slurry to the glass column with the help of a funnel and a glass rod. Tap the column with a glass rod covered with 5 cm rubber tubing. The process of pouring slurry and tapping is continued till a column of about 15 cm deep is formed. Drain off the excess water leaving a water layer of about 5 mm thick above the alumina surface. Wash the column with 10 cm^3 of 0.5 *M* HNO_3 solution. Now cut out a small disc of filter paper (diameter equal to that of glass column) and place it into the column at the top surface of alumina to ensure that the column packing is not disturbed when more liquid is added to the column. The column can now be used for separation. The column is treated with HNO_3 before the chromatography of anions.

   Remove the alumina of the column immediately after chromatography.
2. Take alumina column and clamp it vertically.
3. Pipette accurately 5 cm^3 of the mixture, drip into the column in the form of thin layer of solution at alumina surface, keeping the stopcock closed.
4. Allow to run the mixture evenly into the adsorbent until the liquid level is just above the top of alumina.
5. Add 1 cm^3 of 0.5 *M* HNO_3 and again run the column until the liquid meniscus is just above the top of the column.
6. Develop the column first with 0.5 *M* HNO_3 to elute $KMnO_4$. Collect the fraction of effluent, which is pink, in a conical flask marked as number 1.
7. Next develop the column with 1*M* H_2SO_4 to elute $K_2Cr_2O_7$. Collect the fraction of effluent containing dichromate (light yellow) in a second conical flask (No. 2).
8. Fill in a burette the supplied $KMnO_4$ solution and find its concentration by titrating with the standard (0.05 *M*) ferrous ammonium sulphate solution.
9. Add 10 cm^3 of 0.05 *M* ferrous ammonium sulphate solution to each of the conical flasks 1 and 2. Titrate both solutions separately with standardized K MnO_4 and note the readings.

8.6 OBSERVATIONS

The two components are separated in the form of different coloured zones and are collected in different fractions.

For estimation the results are tabulated in the following manner.

Observation Table-I
Standard Ferrous Ammonium Sulphate Vs Potassium Permanganate

Sl. No.	Volume of ferrous ammonium sulphate in cm^3	Burette Reading Initial	Burette Reading Final	Volume of $KMnO_4$ in cm^3 V_2 (Final–Initial)
1.				
2.				
3.				

Observation Table-II
Effluent Fractions Vs Potassium Permanganate

Flask No.	Volume of $FeSo_4$ $(NH_4)_2SO_4$ added in cm^3 to the flask V_3	Burette Reading Initial	Burette Reading Final	Volume of $KMnO_4$ in cm^3
1..				V_4
2.				V_5

8.6 CALCULATIONS

(a) **Determination of the strength of $KMnO_4$ solution**

Molarity of ferrous ammonium sulphate = M_1 .....

Volume of ferrous ammonium sulphate = V_1 .....

Volume of $KMnO_4$ used = V_2 = ...

Molarity of $KMnO_4$ = M_2 = ?

The redox equation is:

$$5Fe^{2+} + MnO_4^- + 8H^+ \longrightarrow 5Fe^{3+} + Mn^{2+} + 4H_2O$$

$$\frac{M_1V_1}{M_2V_2} = \frac{5}{1}$$

$$M_1 V_1 = 5 M_2 V_2$$

$$M_2 = \frac{M_1 V_1}{5 V_2} = ...M$$

(b) **Determination of the amount of $KMnO_4$ in the sample**

No. of millimoles = Molarity × volume in cm^3.

Millimoles of ferrous ammonium sulphate added = 5 × millimoles of $KMnO_4$ in effluent + 5 × millimoles of $KMnO_4$ used in titration

M_1V_3 = 5 (millimoles of $KMnO_4$ in effluent + $M_2 V_4$)

$$\text{millimoles of } KMnO_4 \text{ in effluent} = \frac{M_1 V_3}{5} - M_2 V_2$$

$$= x$$

Amount of $KMnO_4$ in sample $= x \times 158$ mg

= ..... mg

(c) **Determination of the amount of $K_2Cr_2O_7$ in the sample**

The redox reaction of ferrous ammonium sulphate and $K_2Cr_2O_7$ is:

$$6Fe^{2+} + Cr_2O_7^{2-} + 14H^+ \longrightarrow 2Cr^{3+} + 7H_2O$$

Millimoles of ferrous ammounium sulphate added + 6 × millimoles of $K_2Cr_2O_7$ in effluent + 5 Millimoles of $KMnO_4$ used in titration

M_1V_3 = 6 × millimoles of $K_2Cr_2O_7$ in effluent + 5 M_2 V_5

millimoles of $K_2Cr_2O_7$ in effluent = $M_1V_3 - 5M_2\frac{V_5}{6}$

$= y$

Amount of $K_2Cr_2O_7$ in sample $= y \times 294$ mg.

= .... mg.

8.7 Result and Discussion

Amount of $KMnO_4$ in the sample = ... mg

Amount of $K_2Cr_2O_7$ in the sample = ... mg

In column chromatography of inorganic substances, most commonly used adsorbent is alumina. It is used in neutral, acidic and basic forms. On technical alumina the cations show greater adsorptivity, whereas the anions have lower adsorptivity and travel faster and moved out of the column shortly. The trivalent cations, usually have higher adsorptivity than the divalent and monovalent cations.

If the alumina is treated with HCl or HNO_3 prior to the separation process the anions show greater adsorptivity than the cations. On acidified alumina the anions have been found to be retained in the following order:

$$PO_4^{3-} > Fe(CN)_6^{4-} > Cr_2O_7^{2-} > Cl^- > NO_3^- > MnO_4^- > S^{2-}$$

Though the mechanism of separation on alumina is not completely understood, it may be as follows:

For cations it may be regarded in terms of surface buffering and the interaction of alumina, cations and water. The hydrated cations are attracted to the negative oxygen ends of alumina molecules. Further, the adsorptivity of different cations depends on the size, valency and dipole moment of the aquo cations.

When the acid treated alumina is taken, the exchange of anions with chloride, nitrate or sulphate is responsible for the separation of anions.

For quantitative estimation of the eluted oxidants ($KMnO_4/K_2Cr_2O_7$) with an excess of a standard reducing agent (e.g. $FeSO_4(NH_4)_2SO_46H_2O$) solution. The unreacted reducing agent is then titrated with a standardized oxidizing agent.

EXPERIMENT 9
ESTIMATION OF AMINO GROUPS

Structure

9.1 INTRODUCTION

In Courses CHE-3 (L) and CHE-7 (L) you were introduced to the various techniques for quantitative analysis. We have described different methods which are used in quantitative analysis i.e., gravimetric methods are those in which the weight of a substance is measured, titrimetric methods refer to measurements of a volume, and physiochemical (instrumental) methods are based on the measurement of some physical or chemical property. In the experiments 9 to 14 we will use some of these methods for organic quantitative analysis. In the section 9.2 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 analysis are useful in organic analysis. In section 9.3 though we are giving very a brief introduction to elemental analysis and molecular weight determinations (i.e., determination of empirical and molecular formulae respectively), but we are not giving any experimental details. Because these experiments require the use of more complex or more costly articles of equipment, which are very difficult to provide at graduation level laboratories. After that you will be introduced to the actual experiment in which you will use acetylation and bromination methods for estimation of amino group.

Objectives

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

- describe the significance of organic quantitative analysis in organic analysis,
- determine the amino group in the given sample by acetylation methods,
- determine amino in the given sample by bromination method.
- describe acetylation and bromination phenomena and perforam acid base and iodemetric titrations.

9.2 AN OVERVIEW OF THE ANALYSIS OF ORGANIC COMPOUNDS

The following is an outline of the methods used in the study of organic compounds.

1. 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 commonone for solids is m.p; for liquids, b.p. and more recently infra-red (IR) spectrun 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 testings their purity, are described in the courses 'Chemistry Lab-II'.

(a) 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-OH,CO,-COOH, $-NH_2$ 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 second Block of 'Qualitative Organic Analysis' of course Chemistry Lab-II.

Organic Quantitative Analysis

Having known the constituent elements and the functional groups present in a organic compound, the next important step in its analysis involves quantitative analysis. Permiting the calculations of an empirical formula, which gives the atomic ratio of the elements present. Determination of the relative 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 Spectroscopy (CHE-10). 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, part of the traditional schemes are still quite useful. Also, the required techniques strongly reinforce 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 our laboratories are not equipped with the modern instrumentations.

Now we will concentrate on the organic qualitative analysis.

9.3 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 compounds.

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 mass spectrometry.

ii) Quantitative functional group analysis: This is carried out to find the relative number of the different kinds of functional groups.

9.3.1 Quantitative Elemental Analysis:

The elements commonly found in organic compounds are carbon, hydrogen, oxygen, nitrogen, halogens, sulphurs, phosphorous and metals. The methods used in the determination of the composition by weight of an organic compound are based on simple principle. Most of our undergraduation laboratories do not have the apparatus to conduct practicals of quantitative elemental analysis, therefore, here we are discussing only the principle part.

Carbon, hydrogen and nitrogen: A known weight of the compound is heated to a high temperature in an excess of dry oxygen. The compound burns to form carbondioxide and water. If nitrogen is present in the organic compound, a mixture of nitrogen oxides (and sometimes nitrogen gas) is also produced; the oxides of nitrogen are subsequently reduced by copper to nitrogen. The weights of carbondioxide, water and nitrogen are then found and percentage composition of carbon, hydrogen and nitrogen can be calculated.

Now a day CHN analysis are carried out by analysers known as CHN analyser, which enable the compound to be analysed automatically (see Fig. 9.1)

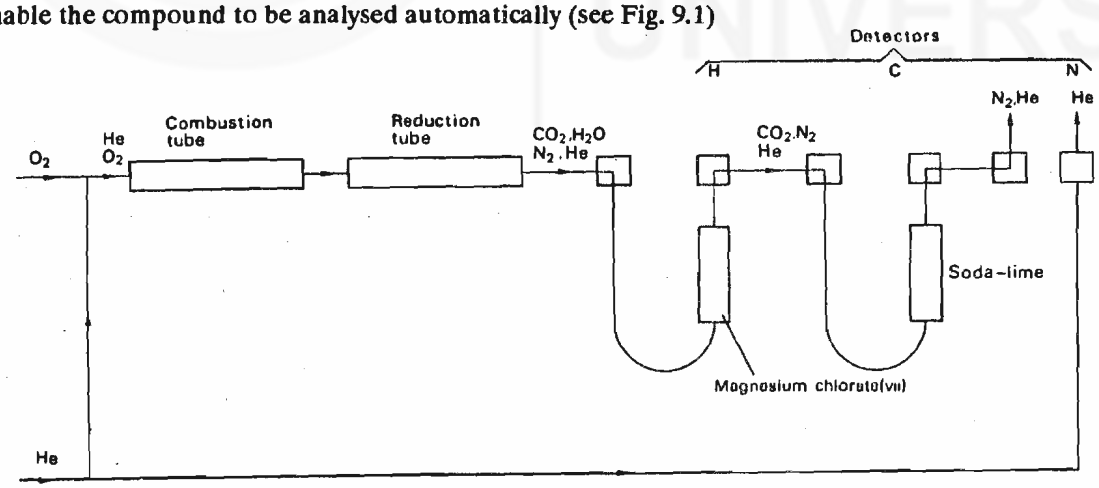


Fig. 9.1: A CHN analyser

Oxygen

It is usually estimated by difference.

Halogens, sulphur and phosphorous

They are usually estimated by the 'oxygen flask' method of combustion. A sample of the compound is wrapped in ashless filter paper and ignited electrically in a flask of

oxygen containing the appropriate absorption liquids for the halogen or sulphur oxides produced. Suitable titration or gravimetric determination gives the amount of these elements present.

Calculation of the Empisical Formula

Once the percentage composition of each element is known, the ratio of the number of atoms of each element present in the compounds can be calculated. This is the **empirical formula.**

The method is to divide the percentage composition of each element by its relative atomic mass and to factorise the resulting numbers so as to obtain simple whole numbers. For example, a compound X, a white solid, was found by analysis to contain 23.30 percent carbon, 8.85 per cent hydrogen and 40.78 percent nitrogen. It was known to contain no other elements, so that the composition of oxygen was 100 – (23.30 + 4.85 + 40.78) = 31.07 per cent then,

Element	% composition	Relative atomic mass	Atomic ratio	Simple atomic ratio
Carbon	23.30	12	$\frac{23.30}{12}=1.94$	2
Hydrogen	4.85	1	$\frac{4.85}{1}=4.85$	5
Nitrogen	40.78	14	$\frac{40.78}{14}=2.91$	3
Oxygen	31.07	16	$\frac{31.07}{16}=1.94$	2

The empirical formula of X is $C_2H_5N_3O_2$. To determine the molecular formula, the relative molecular mass must be found.

Determination of the Molecular Formula

As said above, the molecular formula of a compound (the actual number of each kind of atom in the molecule) can be determined from the empirical formula and the relative molecular mass.

For example, the empirical formula of the compound X in above mentioned example was found to be $C_2H_5N_3O_2$. The formula weight is 103. In this case, therefore, the empirical formula is also the molecular formula. On the other hand, if the relative molecular mass had been found to be 206, the molecular formula would have been $C_4H_{10}N_6O_4$.

The determination of relative molecular masses of compounds is described in detail in 'Physical Chemistry' Course (CHE-04). Those of gases are generally determined by the limiting density method, using a gas density balance, while those for volatile liquids and solids are found by Victor Meyer's method in which the volume of vapour from a known weight of compound is determined. The relative molecular mass of an involatile liquid or solid is often found from the depression of the freezing point of a solvent. The relative molecular mass can also be found quickly and with a very high degree of precision by mass spectrometry. Procedure detail of mass spectrometric method is given in the course 'Spectroscopy' (CHE-09).

9.3.2 Quantitative Functionals 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 grow analysis not only helps to estimate functionals groups presen in a compound but also provides methods for the determination of amount or concentration of organic Constitutents. In this course, you will be introduced to five such experiments. These experiments are the determination of amino groups, hydroxyl groups, sugars, amino acids, and formaldehyde and analysis of oil and fats.

9.4 DETERMINATION OF AMINO GROUPS

In an organic compound amino groups can be estimated by two methods, one of which is based on an acetylation method, while other is based on bromination method. In acetylation method the excess of free acetic acid left after acetylation of amino group is determined by titration with standard sodium hydroxide solution. In bromination method the excess of bromine is determined after bromination of aromatic amines by the addition of potassium iodide solution and titration of liberated iodine with sodium thiosulphate solution. If the molecular weight of the compound is known, the number of amino groups can then be calculated.

Now you will be introduced to the actual experiment in which you will use above mentioned methods for the determination of amino group in a given sample.

9.4.1 Experiment 9a: Determination of Amino Group by Acetylation Method

Principle

The amino group reacts quantitatively with acetic anhydride in presence of base (pyridine) to form acetyl derivative. The excess of acetic anhydride is then hydrolysed with water and the total free acetic acid is found out by titrating with a standard sodium hydroxide solution using phenolphthalein as an indicator. A control or blank experiment is performed (without using amino compound) by taking same amount of acetic anhydride. The difference in the amount of alkali used in the two experiments is equivalent to the acetic acid used in acetylation. If the molar mass of the compound is known, the number of amino groups in the compound can be calculated.

In organic estimation we frequently employ control blank experiment along with original experiment. Such approach has following advantages.

(1) The absolute concentration of a reagent (for example in Experiment 9, the exact amount of acetic anhydride) need not be determined, since if the same amount of reagent is used in the actual and in the control experiments, the difference gives at once the actual amount used.

(2) The losses of the reagents due to the chemical action or the alkaline glass vessels, slight absorption by the curves etc., are almost identical for the actual and the control experiments and therefore, do not affect the difference in result between the two experiments. Ordinary chemical flask, with reflux water condensers, using rubber stopper can also be used.

$$R(NH_2)_n + n(CH_3CO)_2O \xrightarrow{\text{Pyridine}} R(NHCOCH_3)_n + nCH_3COOH$$

$$(CH_3CO)_2O + H_2O \longrightarrow 2CH_3COOH$$

excess acetic acid :

$$CH_3COOH + NaOH \longrightarrow CH_3COONa + H_2O$$

In this experiment pyridine is used as a solvent because it is inactive towards the reagent, it removes the acid products by salt formation, and it also serves as a catalyst.

$$RNH_2 + (CH_3CO)_2O + C_5H_5N \longrightarrow RNHCOCH_3 + (C_5H_5NH)^+ (CH_3COO)^-$$

$$HOH + (CH_3CO)_2O + 2C_5H_5N \longrightarrow 2(CH_3COO)^- + 2(C_5H_5NH)^+$$

Requirements

Apparatus		Chemicals
Burette (50 cm^3)	- 1	Aniline
Conical flask (250 cm^3)		Acetic anhydride
Conical flask (Q.F) (250 cm^3)	- 1	Pyridine
or Round bottom flask (Q.F) 250 cm^3	- 2	Sodium hydroxide
Weighing bottle	- 1	Alcohol
Funnel (small)	- 1	Phenolphthalein
Test-tube	- 1	Soda-lime
Burette stand	- 1	
Water-bath	- 1	
Reflux condenser	- 2	

Solution Provided

i) **Sodium hydroxide solution, 1 *M*:** It is prepared by dissolving 40 g NaOH in distilled water in 1dm^3 flask. This solution can be standardised with either 0.5*M* oxalic acid solution using phenolphthalein indicator.

ii) **Phenolphthalein indicator:** Dissolve 1.0g of phenolphthalein in 100 cm^3 of ethanol and then dilute with 100 cm^3 of water.

Procedure

i) **Preparation of acetylating reagent:** Mix 20 cm^3 of acetic anhydride (AR) and 60 cm^3 of pure and dry pyridine as required in a dry conical flask. Fill the solution in a dry burette.

ii) Take two 250 cm^3 conical flasks (Q.F) marked 'A' and 'B' fitted with reflux condensers. Weigh accurately about 1 g of sample aniline and transfer it to flask 'A'. Then add 10 cm^3 of acetylating reagent to the flask 'A' and also to the blank flash 'B'. Heat the two flasks on boiling water-bath for 45 minutes.

iii) Add 20 cm^3 of distilled water through the condensers in both flasks so that the water rinses down the condenser tube and the walls of the flasks. Shake the contents of the flasks and heat for 2 minutes more. Cool the flasks under running water.

iv) Titrate the contents of each flask separately with 1 *M* sodium hydroxide solution using phenolphthalein as indicator. The difference between the volumes of alkali used in the two titrations corresponds to the anilin which has reacted. Repeat both blank an actual titrations to get at least two concordant readings in each case. Record the observation in Observation Tables I and II for blank and originals titrations; respectively.

Observations

Mass of the weighing bottle	$= m_1$	= .....g
Mass of the bottle+aniline	$= m_2$	= .....g
Mass of the bottle		
(after transferring the compound)	$= m_3$	= .....g
Mass of aniline transferred	$= m_2 - m_3$	$= m =$
Molar mass of aniline	$= M_m$	= 93 g mol^{-1}

Observation Table I
Acetylating Reagent vs. Sodium Hydroxide Solution
(Blank Titration)

Sl. No.	Volume of Acetylating reagent in cm^3	Burette		Volume of NaOH in cm^3 (Final-Initial)
		Initial	Final	
1	10			
2	10			
3	10			

Volume of NaOH used in neutralising 10 cm^3 of acetylating reagent = V_1 =..... cm^3

Pyridine can be dried over KOH

Standardisation of sodium hydroxide solution

Sodium hydroxide is not a primary standard. It is to be standardised. It can be standardised by titrating against standard oxalic acid solution using phenolphthalein as indicator. The procedure is given below:

(1) **Preparation of standard oxalic acid Solution**
Oxalic acid solution, 0.5 *M* : Dissolve 8 g of oxalic acid in water in 250 cm^3 volumetric flask and make up to mark.

(2) **Standardisation of sodium hydroxide solution :** Pipette out 20 cm^3 of standard oxalic acid solution (0.5 M) into a 100 cm^3 conical flask. Add 2-3 drops of phenolphthalein indicator. Titrate with sodium hydroxide solution taken in the burette. Swirl the conical flask after each addition. Continue the titration till a permanent purple colour is obtained as the end point. If M_1 and V_1 are the molarity and volume of oxalic acid solution, whereas M_2 and V_2 are the molarity and volume of sodium hydroxide solution, then the molarity of sodium hydroxide would be given by the following formula (as per stiochiometric equation

$$(COOH)_2 + 2OH^- \longrightarrow 2H_2O + CO_2$$

Molarity of NaOH $= M_2 =$

$$\frac{2M_1V_1}{V_2} = 40\frac{M_1}{V_2}$$

The experiment can also be carried out in two 250 cm^3 conical flask with out testing reflux condensers; since very little evaporation of the acetylating mixture, from an open conical flask would occur during heating on the water-bath.

Observation Table II
Sample + Acetylating Reagent vs. Sodium Hydroxide Solutio
(Actual Titration)

Sl. No.	Sample + 10 cm^3 Acetylating reagent in cm^3	Burette		Volume of NaOH in cm^3 (Final-Initial)
		Initial	Final	
1				
2				
3				

Volume of sodium hydroxide used in neutralising the

sample +10 cm^3 of acetylating reagent $= V_2$ =..... cm^3

Calculations

Mass of the sample $= m$ g =..... g

Difference of sodium hydroxide

Solution required for OT-I & OT-II $= V_1 - V_2$ cm^3=..... cm^3

1000 cm^3 M_2 NaOH = M_2 g mol. NaOH = M_2 g mol. CH_3COOH = M_2 g mole NH_2, where M_2 = molarity of sodium hydroxide solution

$$(V_1 - V_2) \text{ cm}^3 \text{ of } M_2 \text{ NaOH} = \frac{M_2 \times (V_1 - V_2)}{1000} \text{ g mol. of } NH_2$$

or
$$= \frac{16 \times M_2 \times (V_1 - V_2)}{100} \text{ g of } NH_2$$

As you know, this is due to m g of sample, therefore, for 100 gms of the sample you will have (% of the NH_2) group

$$\% \text{ } NH_2 = \frac{16 \times M_2 \times (V_1 - V_2) \times 100}{m \times 1000}$$

=..... %

ii) The number of amino group (NH_2) in the sample (aniline) can be calculated as follows:

From above

$$\text{m g of sampl} \equiv \frac{16 \times M_2 \times (V_1 - V_2)}{1000} \text{g of } NH_2 \text{ group}$$

$$\text{93 g (1 g mol) of amine contain} = \frac{16 \times M_2 \times (V_1 - V_2) \times 93}{1000 \times m} NH_2 \text{ group}$$

Since 16.03 g mass is due to the one NH_2 group

$$\text{Therefore, aniline contains} = \frac{16 \times M_2 \times (V_1 - V_2) \times 93}{1000 \times m \times 16} NH_2 \text{ group (s)}$$

=..... NH_2 group(s)

Result

The percentage of amino group in the sample of aniline =..... %

The number of amino group(s) in the sample of aniline = ...

9.4.2 Experiment 9b: Determine Aniline by Bromination Method

Principle

Aniline and some of its derivatives having free *ortho* and *para* positions can be estimated by bromination method. The method involves the following steps:

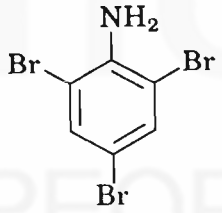
(a) **Bromination of aniline by bromination mixture:**

Aniline reacts with bromine to form 2, 4, 6– tribromoaniline. Since the yield is quantitative, it is used for the estimation of aniline. The bromine required is obtained by treating a mixture of potassium bromide and potassium bromate with dilute hydrochloric acid. The bromine so liberated reacts with aniline to produce tribromoaniline while excess of bromine remains unreacted.

$$5\,KBr + KBrO_3 + 6HCl \longrightarrow 6KCl + 3Br_2 + 3H_2O$$

The number of bromine molecule consumed by various phenols is given against the name of the phenol given below:

Phenol - three,
Cresol - three,
o- and *p*- Nitrophenol - two,
m-Nitrophenol - three,
Resorainol - three,
Salicylic acid - three,
Naphthol - one.



2, 4, 6-Tribromoaniline

(b) **Determination of unreacted bromine:**

The unreactive bromine is treated with potassium iodide, the equivalent iodine thus liberated is determined iodometrically with sodium thiosulphate (hypo) solution using starch as indicator.

$$2KI + Br_2 \longrightarrow 2KBr + I_2$$

$$I_2 + 2Na_2S_2O_3 \longrightarrow Na_2S_4O_6 + 2NaI$$

Sodium tetrathionate

Requirements

Apparatus		Chemicals
Burette (50 cm^3)	- 1	Aniline (AR)
Pipette (25 cm^3)	- 1	Potassium bromide (AR)
Conical flask (250 cm^3)	- 1	Potassium bromate anhydrous (AR)
Vol. flasks (250 cm^3)	- 2	Potassium iodide (AR)
		Sodium thiosulphate
Weighing bottle	- 1	conc. hydrochloric acid
Funnel (small)	- 1	
Test-tube	- 1	

Wash-bottle for dist. water - 1

Burette stand - 1

Solutions Provided:

i) **Sodiumthiosulphate solution 0.1*M*:** It is prepared by dissolving 6.25g of sodium thiosulphate pentahydrate in 250 cm^3 distilled water in a volumetric flask.

ii) **Potassium iodide soltuion, 20 per cent:** Dissolve 20g of A.R. potassium iodide in 100 cm^3 of distilled water.

iii) **Starch indicator solution:** Make a paste of 1.0g of starch with a little water and pour the suspension, with constant stirring into 100 cm^3 of boiling water.

Procedure

1. **Preparation of Brominating solution (0.2 *M*):** Weigh 1.4 gm of A.R. potassium bromate and 9 gm of potassium bromide A.R. in water and make up the volume to 250 cm^3 in a volumetric flask. Fill the solution in a burette.

2. **Preparation of standard solution aniline:** Weigh accurately about 0.5 g aniline in a weigh bottle. Transfer this to a 250 cm^3 volumetric flask. Weigh the weighing bottle again and find the exact mass of aniline transferred by difference. Dissolve it in water and make up the volume to 250 cm^3.

3. **Titration with brominating solution (Blank titration)**
Pipette out 25 cm^3 of brominating solution in a 250 cm^3 conical flask and add 25 cm^3 of distilled water, 5 cm^3 of concentrated hydrochloric acid and 5 cm^3 of KI solution. Shake the contents of the conical flask, the solution will become dark-brown due to liberation of iodine. Titrate this with sodium thiosulphate solution until the solution acquires light yellow colour and then add 5-6 drops of starch solution and continue the titration with sodium thiosulphate solution carefully. At the end point blue colour disappears. Repeat the titration to get at least two concord readings to ensure a correct and exact measurement. Record the observations in observation Table I. This titration is used to determine the volume of brominating solution which is equivalent to 1 cm^3 of sodium thiosulphate solution.

4. **Titration with standard aniline solution**
Pipette out 25 cm^3 of standard aniline solution in a 250 cm^3 conical flask and add 25 cm^3 of distilled water and 5 cm^3 concentrated HCl. Brominating mixture (taken in burette) is now added to this solution till it achieves light yellow in colour and then add 5 cm^3 of KI solution. Liberated iodine is titrated against sodium thiosulphate solution using starch as an indicator. Repeat the titration to get at least two concordant readings. Records the observation in Observation Table II

5. **Titration with unknown aniline solution (Actual titration):**
Take out 25 cm^3 of unknown aniline solution in a 250 cm^3 conical clask and titrate similarly as described in case of standard aniline solution. Repeat titration to get at least two concerdant reading. Record the observations in Observation Table III.

Volume of brominating mixture used in each set of titration should preferably be same.

Observations

Mass of the weighing bottle $= m_1$ =..... g

Standardised of sodium thiosulphate solution

Sodium thiosulphate ($Na_2S_2O_3.5H_2O$) is not a primary standard. It has to be standardised. It can be standardised by titrating against standard potassium dichromate solution iodometrically using starch as indicator. The procedure is given below:

1) **Preparation of standard dichromate solution** The dichromate solution can be prepared by weighing accurately about 1.226 g $K_2Cr_2O_7$, dissolving in water and making up to 250 cm^3 in a standard flask.

2) **Standardisation of sodium thiosulphate solution** Into a 250 cm^3 conical flask, pipette 20 cm^3 of standard potassium dichromate solution (0.016 *M*). Add 10 cm^3 of 1 M sulphuric acid and 1 g of sodium hydrogen carbonate into the conical flask with gentle swirling. Then add 0.5 g potassium iodide or 10 cm^3 of 5% KI solution, swirl, cover the flask with watch glass and allow the solution to stand for 5 minutes in a dark place. Titrate against sodium thiosulphate solution taken in the birutte, until a light pale yellow colour is obtained. Add 2 cm^3 of starch solution and continue the titration till the blue colour of starch iodine complex disappears. If M_1 and V_1 are the molarity and the volume of $K_2Cr_2O_7$ used whereas M_2 and V_2 are the molarity and volume of thiosulphate required for titration, then the molarity of $Na_2S_2O_3$ solution could be obtained as follows:

As per the stoichiometric equation

$$Cr_2O_7^{2-} + 14H^+ + 6S_2O_3^{2-} \longrightarrow 2Cr^{3+} + 3S_4O_6^{2-} + 7H_2O =$$

$$\frac{M_1V_1}{M_2V_2} = \frac{1}{6}$$

$$\text{or } M_2 = \frac{6M_1V_1}{V_2}$$

or Molarity of thiosulphate solution $= M_2$

$$= 120\frac{M_1}{V_2}$$

(since $V_1 = 20\,cm^3$)

Mass of the bottle + aniline $= m_2$ =..... g

Mass of the bottle

(after transferring the aniline) $= m_3$ =..... g

Mass of aniline transferred $= m_2 - m_3 = m$ =..... g

Molar mass (M_m) of aniline = 93 g mol^{-1}

Observation Table I
Brominating solution vs. Sodium thiosulphate Solution
(Blank Titration)

Sl. No.	Volume of brominating solution in cm^3	Burette reading		Volume of $Na_2S_2O_3$ solution in cm^3 (Final-Initial)
		Initial	Final	
1	25			
2	25			
3	25			

Observation Table II
Standard Aniline Solution vs. Sodium Thiosulphate Solution

Sl. No.	Volume of aniline solution in cm^3	Volume of Brominating solution in cm^3	Burette reading		Volume of $Na_2S_2O_3$ solution in cm^3 (Final-Initial)
			Initial	Final	
1	25				
2	25				
3	25				

Observation Table III
Unknown Aniline Solution vs. Sodium Thiosulphate Solution
(Actual titration)

Sl. No.	Volume of unknown aniline solution in cm^3	Volume of Brominating solution in cm^3	Burette reading		Volume of $Na_2S_2O_3$ solution in cm^3 (Final-Initial)
			Initial	Final	
1	25				
2	25				
3	25				

Calculations:

i) Mass of aniline in standard solution = m g

ii) Volume of sodium thiosulphate used in blank experiment against 25 cm^3 brominating solution = V cm^3 =..... cm^3

iii) Volume of sodium thiosulphate used for standard aniline solution = V_1 cm^3 =..... cm^3

iv) Volume of sodium thiosulphate used for unknown aniline solution = V_2 cm^3 =..... cm^3

V cm^3 of sodium thiosulphate solution =25 cm^3 brominating solution

Hence 1 cm^3 of sodium thiosulphate = $\frac{25}{V}$ cm^3 brominating solution

Therefore, V_1 cm^3 of sodium thiosulphate

$$= \frac{25}{V} \times V_1 \text{ cm}^3 \text{ brominating solution}$$

Hence, volume of brominating solution used for 25 cm^3 of standard aniline solution

$$= V - \frac{25}{V} \times V_1 \text{ cm}^3 = V_3 \text{ cm}^3$$

Similarly, volume of brominating solution used for 25 cm^3 of unknown aniline solution

$$= V - \frac{25}{V} \times V_2 \text{ cm}^3 = V_4 \text{ cm}^3$$

Using relation,

$$\frac{\text{mass of aniline in unknown solution}}{\text{mass of aniline in standard solution}} = \frac{\text{volume of brominating solution used in unknown aniline solution}}{\text{volume of brominating solution used in standard aniline solution}}$$

$$\text{mass of aniline in unknown solution} = \frac{m \times V_4}{V_3} = \ldots \text{ g per } 250 \text{ cm}^3$$

$$\text{The strength of aniline in unknown solution} = \frac{\text{Strength of standard aniline solution} \times V_4}{V_3}$$

$$= \frac{4 \times m \times V_4}{V_3} = \ldots\ldots\ldots\ldots\ldots\ldots \text{g dm}^{-3}$$

Result

The amount of aniline in given unknown solution is g.

The strength of aniline in given unknown solution g dm^{-3}.

The percentage purity of aniline can also be calculated by the following formula:

$$\% \text{ purity of aniline} = \frac{(V - V_2) \times M \times M_m \times 100}{m \times z \times 2000}$$

where,

V = volume of sodium thiosulphate used in blank experiment.

V_2 = volume of sodium thiosulphate used in sample of aniline.

M = Molarity of sodium thiosulphate solution.

M_m = Molar mass of aniline.

m = mass of aniline taken in g

z = number of bromine atoms substituted in aniline.

EXPERIMENT 10
ESTIMATION OF PHENOLS

Structure

10.1 INTRODUCTION

Similar to amine groups, hydroxyl groups can also be estimated by two methods. One of which is based on an acetylation method, while other is based on bromination method. In acetylation method, the excess of free acetic acid left after acetylation of hydroxyl group is determined by titration with standard sodium hydroxide solution. In bromination method, the excess of bromine is determined after bromination of hydroxyl compounds by the addition of potassium iodide solution and titration of liberated iodine with sodium thiosulphate solution. If the molecular weight of the compound is known, the number of hydroxyl groups can then be calculated.

Objectives

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

- determine the amount of phenol in the given sample,
- describe acetylation and bromination methods, and
- perform acid-base and iodimetric titrations

10.2 EXPERIMENT 10 a: DETERMINATION OF HYDROXYL GROUP BY ACETYLATION METHOD

10.2.1 Principle

In Experiment 9a, you have estimated the amino group by acetylation method. This method can also be used to determine phenol or hydroxyl groups (alcohols). In this experiment, we will determine the number of hydroxyl groups in a phenol.

$$ArOH + (CH_3CO)_2 \xrightarrow{\text{Pyridine}} ArOC\text{-}CH_3 + CH_3COOH$$

phenol	acetic anhydride	acetyl deriv.	acetic acid

In this experiment pyridine is used as a solvent because it is inactive towards the reagents, it removes the acid products by salt formation, and it also serves as a catalyst.

$$ROH + (CH_3CO)_2 + C_6H_5N \longrightarrow ROCOCH_3 + (C_5H_5NH)^+(CH_3COO)^-$$

$$HOH + (CH_3CO)_2O + 2C_5H_5N \longrightarrow 2CH_3COO + 2C_5H_5NH^+$$

10.2.2 Requirements

You can use the same apparatus, chemicals and solution, which you have prepared for Experiment 9a. But in this experiment, in place of aniline you will use phenol.

10.2.3 Procedure

Similar to Experiment 9a, first prepare acetylating reagent. Then take two conical flask, A and B, fitted with reflux water condensers. Weigh accurately about 1 g of phenol and transfer in flask A. Add 10 cm^3 of acetylating reagent to the both flasks A and B. Connect the flasks to the reflux condensers and heat both flasks on boiling water-baths for 30 minutes. Then remove both the flasks from water-bath and pour 20 cm^3 of distilled water down each condenser, shaking the contents of each flask gently to ensure complete hydrolysis of the unreacted acetic anhydride. Finally cool each flask thoroughly in cold water and allow it to stand for 10 minutes. Then titrate the contents of each flask with *M* NaOH solution, using phenolphthalein or an indicator. A fine emulsion of phenyl acetate will form when the contents of the flask A are diluted and should therefore be vigorously stirred throughout the titration to ensure that all the free acetic acid is extracted by the sodium hydroxide solution. Repeat both blank and actual titrations to get at least two concordant readings. Record the observations in Observation Tables I & II for blank and original titrations, respectively.

10.2.4 Observations

Mass of the weighing bottle $= m_1$ =..... g

Mass of the bottle + phenol $= m_2$ =..... g

Mass of the bottle

(after transferring the compound) $= m_3$ = ...g

Mass of phenol transferred $= m_2 - m_3$ $= m$ =

Molar mass of phenol $= M_m$ $= 94\ g\ mol^{-1}$

Observation Table I
Acetylating Reagent vs. Sodium Hydroxide Solution
(Blank titration)

Sl. No.	Volume of Acetylating reagent in cm^3	Burette		Volume of NaOH in cm^3 (Final–Initial)
		Initial	Final	
1	10			
2	10			
3	10			

Volume of NaOH used in neutralising 10 cm^3 of Acetylating reagent = V_1 =..... cm^3

Observation Table II
Sample + Acetylating Reagent vs. Sodium Hydroxide Solution
(Actual titration)

Sl. No.	Sample + 10 cm^3 Acetylating reagent in cm^3	Burette		Volume of NaOH in cm^3 (Final–Initial)
		Initial	Final	
1				
2				
3				

Volume of sodium hydroxide used in neutralising the sample + 10 cm^3 of acetylating reagent = V_2 = cm^3 ..... cm^3

10.2.5 Calculations

Mass of the sample $= m$ = ... g

Difference of sodium hydroxide

Solution required for OT-I & OT-II $= V_1 - V_2$ cm^3 = ... cm^3

1000 cm^3 M_2 NaOH = M_2 g mol. NaOH ≡ M_2 g mol. CH_3COOH ≡ M_2 g mol. OH
where M_2 = molarity of sodium hydroxide solution.

$$(V_1 - V_2)\ cm^3 \text{ of } M_2 \text{ NaOH} = \frac{M_2 \times (V_1 - V_2)}{1000} \text{ g mol. of OH}$$

$$\text{or} = \frac{17 \times M_2 \times (V_1 - V_2)}{100} \text{ g of OH}$$

As you know, this is due to m g of sample, therefore, for 100 gms of the sample you will have (% of the OH_2) group

$$\% \text{ OH} = \frac{17 \times M_2 \times (V_1 - V_2) \times 100}{m \times 1000}$$

.....%

ii) The number of hydroxy group (OH) in the sample (phenol) can be calculated as follows:

From above

$$\text{m g of sample} \equiv \frac{17 \times M_2 \times (V_1 - V_2)}{1000} \text{ g of OH group}$$

$$\text{94 g (1 g mol) of sample contain} = \frac{17 \times M_2 \times (V_1 - V_2) \times 94}{1000 \times m}$$

Since 16.03 g mass is due to the one OH group

$$\text{Therefore phenol contain } \frac{17 \times M_2 \times (V_1 - V_2) \times 94}{1000 \times m \times 17} \text{ OH group(s)}$$

$$= \frac{M_2 \times (V_1 - V_2) \times .094}{m} \text{ OH group(s)}$$

=..... OH group(s)

10.2.6 Result

% of OH group in the sample of phenol =..... %

The number of OH group in the phenol = ...

10.3 EXPERIMENT 10b: DETERMINATION OF PHENOL BY BROMINATION METHOD

10.3.1 Principle

Bromination with brominating reagents (a mixture of potassium bromide, potassium bromate and conc. HCl) as described in case of Experiment 9 b, can also be employed for determination of phenol.

$$5KBr + KBrO_3 + 6HCl \longrightarrow 3Br_2 + 3H_2O + 6KCl$$

The excess of bromine is determined by the addition of potassium iodide solution and titration of the liberated iodine with standard thiosulphate solution.

$$Br_2 + 2KI \longrightarrow 2KBr + I_2$$

$$2Na_2S_2O_3 + I_2 \longrightarrow 2NaI + Na_2S_4O_6$$

10.3.2 Requirements

You can use the apparatus, chemicals and solution, which you have prepared for Experiment 9 b. But in this experiment in place of aniline you will use phenol.

10.3.3 Procedure

1. First prepare brominating solution as described for Experiment 9 b and then prepare standard solution of phenol by dissolving accurately weighed phenol (about 0.4 g) in water in 250 volumetric flask.

2. **Titration with brominating solution (Blank titration):** In this experiment you are using same brominating solution which you have used for the experiment 9 b. Therefore, there is no need to repeat this step.

3. **Titration with standard phenol solution:** Pipette out 25 cm^3 of standard phenol solution in a 250 cm^3 conical flask and add 25 cm^3 of distilled water and 5 cm^3 concentrated HCl. Brominating mixture (taken in burette) is now added to this solution till it achieves light yellow colour and then add 5 cm^3 of KI solution. Liberated iodine is titrated against sodium thiosulphate solution using starch as an indicator. Repeat the titration to get at least two concordant readings. Record the observations in Observation Table-I.

4. **Titration with unknown phenol solution:** Take out 25 cm^3 of unknown phenol solution in 250 cm^3 conical flask. treat it as described in case of standard phenol solution and then titrate similarly. Repeat the titration to get at least two Concordant readings. Record the observations in Observation Table II.

10.3.4 Observation

Mass of the weighing bottle $= m_1$ =..... g

Mass of the bottle + phenol $= m_2$ =..... g

Mass of the bottle $= m_3$ =..... g

(after transferring the phenol)

Mass of phenol transferred $= m_2 - m_3 = m_3$ =..... g

Molar mass (Mm) of phenol = 94 g mol^{-1}.

Observation Table - I
Standard Phenol Solution vs. Sodium Thiosulphate Solution

Sl. No.	Volume of phenol solution in cm^3	Volume of brominating solution in cm^3	Burette reading		Volume of $Na_2S_2O_3$ solution in cm^3 (Final–Initial)
			Initial	Final	
1	25				
2	25				
3	25				

Observation Table II
Unknown Phenol Solution vs. Sodium Thiosulphate Solution

Sl. No.	Volume of unknown phenol solution in cm^3	Volume of Brominating solution in	Burette reading		Volume of $Na_2S_2O_3$ solution in cm^3 (Final–Initial)
	cm^3		Initial	Final	
1	25				
2	25				
3	25				

10.3.5 Calculation

i) Weight of phenol in standard solution $= m$ =..... g

ii) Volume of $Na_2S_2O_3$ solution in blank experiment against brominating solution (from Observation Table - I of Experiment 9b) $= V$ =..... cm^3

iii) Volume of sodium thiosulphate used for standard phenol solution (from Observation Table I) $= V_1$ =..... cm^3

iv) Volume of sodium thiosulphate used for unknown phenol solution (from Observation Table II) $= V_2$ =..... cm^3

$V cm^3$ of sodium thiosulphate solution $= 25 cm^3$ brominating solution

Hence 1 cm^3 of sodium thiosulphate $= \frac{25}{V}$ cm^3 brominating solution

Therefore, V_1 cm^3 of sodium thiosulphate $= \frac{25}{V} \times V_1$ cm^3 brominating solution

Hence, volume of brominating solution used for 25 cm^3 of standard phanol solution

$$= \frac{V-25}{V} \times V_3 \text{ cm}^3$$

Similarly, volume of brominating solution used for 25 cm^3 of unknown solution

$$= V - \frac{25}{V} \times V_2 \text{ cm}^3 = V_4 \text{ cm}^3$$

using relation

$$\frac{\text{mass of phenol in known solution}}{\text{mass of phenol in standard solution}} =$$

$$\frac{\text{Volume of brominating solution used in unkown phenol solution}}{\text{Volume of brominting solution used in standard phenol solution}}$$

Weight of phenol in unknown solution $= m \times \frac{V_4}{V_3}$

$= \ldots\ldots\ldots\ldots$ g

Strength of phenol in unknown solution $= \frac{\text{Strength of standard phenol solution} \times V_4}{V_3}$

$= \frac{4 \times m \times V_4}{V_3}$

$= \ldots\ldots\ldots\ldots$ g dm^{-3}

10.3.6 Result

The amount of phenol in unknown solution = ... g

The strength of phenol in unknown solution = ... g dm^{-3}

The percentage purity of phenol can also be calculated by the following formula

$$\% \text{ of purity of phenol} = \frac{(V - V_2) \times M \times M_m \times 100}{M \times z \times 2000}$$

where,

V = Volume of sodium thiosulphate used in blank experiment

V_2 = Volume of sodium thiosulphate used in sample of phenol

M = Molarity of sodium thiosulphate

M_m = Molar mass of phenol

m = Mass of phenol taken in g

z = number of bromine atoms substituted in phenol

Experiment 11
ESTIMATION OF SUGARS

Structure.

11.1 INTRODUCTION

Two methods are in common use for the estimation of sugars.

(i) Chemical, depending upon the reducing properties of certain sugars

(ii) Polarimetric, depending upon the optical activity of the sugars concerned.

The second method is most accurate and rapid method, and is of considerable technical importance. The chemical method, although less accurate than the polarimetric method, is of great value for the estimation of sugars in biological fluids.

Using chemical methods, the reducing sugars like glucose and fructose may be estimated quantitatively be oxidising agents like

(i) Benedict's solution

ii) Fehling's solution

Now, we will perform two experiments using Benedict's solution and Fehling's solution.

Objectives

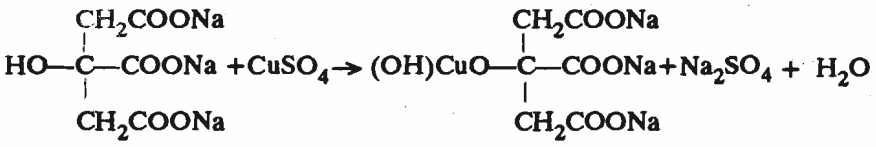
After studying and performing this experiment, you should be able to

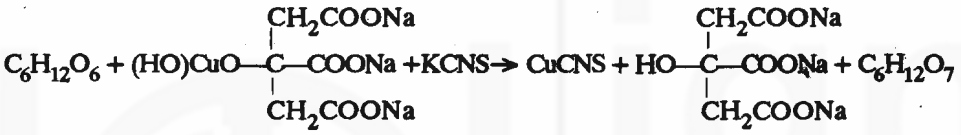
- determine the amount of glucose in the given sample,
- describe oxidation reactions of reducing sugars with Benedicts and Fehling's solutions, and
- perform redox titrations using standard solution of Benedict's and Fehling's Solutions.

11.2 EXPERIMENT 11a: DETERMINATION OF GLUCOSE BY BENEDICT'S SOLUTION

11.2.1 Principle

Glucose readily reduces Benedict's solution, which is an alkaline solution of cupric ions. It is prepared by dissolving copper sulphate, sodium carbonate, sodium citrate, potassium thiocyanate and potassium ferrocyanide in proper proportions in distilled water. Sodium citrate prevents the precipitation of cupric hydroxide by forming a complex, while potassium thiocyanate is used to precipitate copper ions as copper thiocyanate.





Glucose | Cooper thiosulphate (white ppt.) | Gluconic acid

11.2.2 Requirements

Apparatus		Chemicals
Burette (50 cm^3)	- 1	Glucose (AR)
Pipette (25 cm^3)	- 1	Sodium carbonate
Conical flask (250 cm^3)	- 1	Sodium citrate
Vol. flasks (250 cm^3)	- 1	Copper sulphate (AR)
Beaker (250 cm^3)	- 1	Potassium thiocyanate
Weighing bottle	- 1	Potassium ferrocyanide
Funnel (small)	- 1	
Wash-bottle for distilled water	- 1	
Burette stand	- 1	

Solution Provided:

Benedict's solution: i) Dissolve 4.5 g of copper sulphate in 20 cm^3 of distilled water.

ii) Dissolve 50 g of crystalline sodium carbonate in 175 cm^3 boiling distilled water. To the clear boiling solution add 50 g of crystalline sodium citrate, when sodium citrate has been completely dissolved then add 32 g of potassium thiocyanate and boil till a clear solution is obtained.

iii) Mix the solutions (i) and (ii) in a 250 cm^3 volumetric flask and add 2 cm^3 of 5% potassium ferrocyanide solution and make up the total volume to 250 cm^3.

If the above quantities are weighed accurately then, 25 cm^3 of the Benedict's a solution = 0.05 g of glucose

11.2.3 Procedure

i) **Preparation of Standard glucose solution:** Weigh accurately about 1.25 g of glucose and transfer to a 250 cm^3 volumetricflask. Dissolve it in small quantity of distilled water and make up to the mark.

ii) **Titration with standard glucose solution:** Fill the burette with the standard glucose solution. Now pipette out 25 cm^3 of Benedict's solution in a 250 cm^3 conical flask and add 3 g of anhydrous sodium carbonate to it. Boil the contents of the conical flask and add gradually the glucose solution with continuous shaking till the blue colour of the solution just disappears and white precipitate of CuCNS begins to appear. Keep the conical flask on burner during the addition of glucose solution. Repeat the process to get atleast two concordent. Record reading the observation in Observations Table I.

iii) **Titration with unknown solution:** Repeat the above procedure with unknown glucose solution. Records the observation in Observation Table II.

11.2.4 Observations

Mass of the weighing bottle $= m_1$ =..... g

Mass of the bottle + glucose $= m_2$ =..... g

Mass of the bottle

(after transferring the compound) $= m_3$ =..... g

Mass of glucose transferred $= m_2 - m_3 = m$ =..... g

Observation Table I
Standard Glucose Solution Vs. Benedict's Solution

Sl. No.	Volume of Benedict's solution in cm^3	Burette reading		Volume of glucose solution in cm^3 (Final-Initial)
		Initial	Final	
1	25			
2	25			
3	25			

Observation Table II
Unknown Glucose Solution Vs. Benedict's Solution

Sl. No.	Volume of Benedict's solution in cm^3	Burette reading		Volume of glucose solution in cm^3 (Final-Initial)
		Initial	Final	
1	25			
2	25			
3	25			

11.2.5 Calculations

The volume of standard glucose used for titrating 25 cm^3 of Benedict's solution $= V_1$ cm^3

The volume of unknown glucose used for titrating 25 cm^3 of Benedict's solution $= V_2$ cm^3

The amount of glucose in the unknown glucose solution $= \frac{m \times V_1}{V_2} =$..... g

The strength of the unknown glucose solution

$$= \frac{\text{strength of standard glucose solution} \times V_1}{V_2}$$

$$= \frac{4 \times m \times V_1}{V_2} = \text{g dm}^{-3}$$

11.2.6 Result

The amount of glucose in the given solution =..... = g

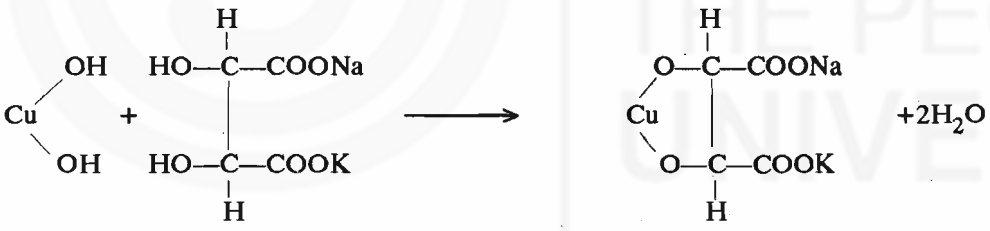
The strength of the unknown glucose solution =..... = g dm^{-3}

11.3 EXPERIMENT 11b: DETERMINATION OF GLUCOSE WITH THE HELP OF FEHLING'S SOLUTION

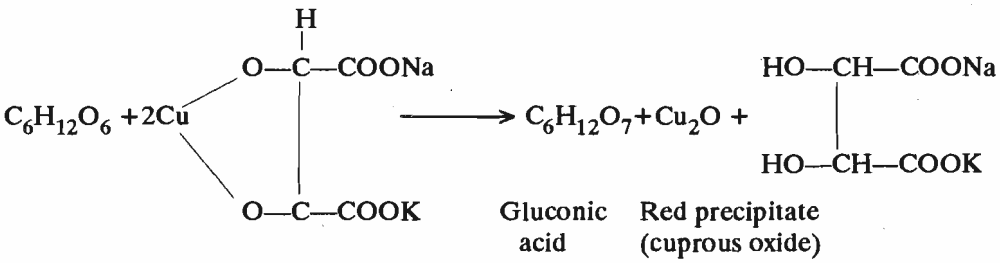
11.3.1 Principle

Glucose also reduces Fehling's solution quickly, the latter is obtained by mixing an aqueous solution of copper sulphate with alkaline solution of sodium potassium tartarate (Rochelle salt). The latter prevents the precipitation of cupric hydroxide by forming a complex. A freshly prepared Fehling's solution is first standardised by titration against a standard glucose solution. The standardised Fehling's solution is then used to determine the amount of glucose in an unknown sample.

$$CuSO_4 + 2NaOH \longrightarrow Cu(OH)_2 + Na_2SO_4$$



Rochelle salt — Soluble complex



Gluconic acid — Red precipitate (cuprous oxide)

11.3.2 Requirements

Apparatus		Chemicals
Burette (50 cm^3)	- 1	Glucose
Pipette (10 cm^3)	-1	Copper sulphate
Conical flask (250 cm^3)	- 1	Sodium hydroxide
Vol. flasks (250 cm^3)	- 1	Sodium Potassium

Beaker (250 cm^3)	- 1	tartarate
Weighing bottle	- 1	Methylene blue
Funnel (small)	- 1	
Wash-bottle for	- 1	
distilled water		
Burette stand	- 1	

Solution Provided:

i) **Fehling's solution A:** Dissolve 17.32 g of crystalling copper sulphate in distilled water. Transfer the solution in 250 cm^3 volumetric flask, wash the container with distilled water, transfer the washing to volumetric flask and make up to the mark.

ii) **Fehling's solution B:**
Dissolve 86.5 g of sodium potassium tartarate (Rochelle salt) and 30 g of sodium hydroxide in warm water, cool and transfer the solution in 250 cm^3 volumetric flask and make up to the mark.

iii) **Methylene blue indicator:** Prepare 1% aqueous solution of methylene blue.

11.3.3 Procedure

i) **Preparation of Standard solution of glucose:** i) Similar to Experiment 11a, dissolve accurately weighed quantity of glucose about 1.25 g in distilled water and make up to 250 cm^3 in a volumetric flask.

ii) **Titration with standard glucose solution:** Fill the burette with standard glucose solution. Pipette out 10 cm^3 each of Fehling's solution A and B in a 250 cm^3 conical flask and dilute it with 25 cm^3 water. Boil the solution gently over a wire gauze and titrate with standard glucose solution, (taken in burette) adding 1 cm^3 of the solution at a time till the blue colour of the solution just disappears and red precipitate of Cu_2O begins to appear. Continue the heating of the Fehling's solution during the titration. Repeat the process to get atleast two concordant readings. Record the observations in Observation Table I.

iii) **Titration with unknown glucose solution:** Now take unknown glucose solution in burette and repeat the titration exactly in the same manner as described for standard glucose solution. To detect the end point more accurately add 4-5 drops of methylene blue indicator to the Fehling's solution just before the end point. The disappearance of the colour will give exact and point. Record the Observations in Observation Table II.

11.3.4 Observations

Mass of the weighing bottle	$= m_1$	= ..... g
Mass of the bottle + glucose	$= m_2$	= ..... g
Mass of the bottle		
(after transferring the compound)	$= m_3$	= ..... g
Mass of glucose transferred	$= m_2 - m_3 = m$	= ..... g

Observation Table I
Standard Glucose Solution vs. Fehling's Solution

Sl. No.	Volume of Fehling's solution in cm^3	Burette reading		Volume of glucose solution in cm^3 (Final–Initial)
		Initial	Final	
1	20			
2	20			
3	20			

Observation Table II
Unknown Glucose Solution vs. Fehling's Solution

Sl. No.	Volume of Fehling's solution in cm^3	Burette reading		Volume of glucose solution in cm^3 (Final–Initial)
		Initial	Final	
1	20			
2	20			
3	20			

11.3.5 Calculations

The volume of standard glucose solution used for titrating 20 cm^3 of Fehling's solution $= V_1$ cm^3

The volume of unknown glucose solution used for titrating 20 cm^3 of Fehling's solution $= V_2$ cm^3

The amount of glucose in the unknown glucose solution $= m \times \frac{V_1}{V_2} = \ldots\ldots$ g

$$\text{The strength of the unknown glucose solution} = \frac{\text{Strength of standard glucose solution} \times V_1}{V_2}$$

$$= \frac{4 \times m \times V_1}{V_2} = \ldots\ldots \text{g dm}^{-3}$$

11.3.6 Result

The amount of glucose in the given solution = ...g

The strength of the unknown glucose solution = ... g dm^3

Experiment 12
ESTIMATION OF AMINO ACIDS

Structure

12.1 INTRODUCTION

There are many titrimetric methods available for the determination of amino acids. Here we will estimate glycine by formal titration method (Soronsen's method).

Objectives

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

- determine the amount of glycine in the given sample,
- describe formylation reaction, and
- perform acid–base titration using standard alkali

12.2 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 alkalies the dissociation favours formation of acid ion.

$$H_2N - CH_2COOH \rightleftharpoons N^+H_3 - CH_2COO^-$$

$$N^+H_3CH_2COO^- + OH^- \rightleftharpoons NH_2CH_2COO^- + H_2O$$

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.

$$CH_2 = O + H_2NCH_2COO^- \longrightarrow CH_2 = NCH_2COO^- + H_2O +$$

$$CH_2OHNHCH_2COO^- + (CH_2OH)_2NCH_2COO^- + \text{etc.}$$

12.3 REQUIREMENTS

Apparatus		Chemicals
Burette (50 cm^3)	- 1	Glycine

Pipette (25 cm^3)	- 1	Sodium hydroxide	
Vol. flasks (250 cm sup 3)	- 1	Formalin solution	
Conical flask (250 cm sup 3)	- 1	Phenolphthalein indicator	
Weighing bottle	- 1		
Funnel (small)	- 1		
Wash-bottle for	- 1		
distilled water			
Test-tube	- 1		
Burette stand	- 1		

Solutions Provided

ii) **Sodium hydroxide solution. O.1*M*:** Dissolve 2 g of sodium hydroxide in a 250 cm^3 volumetric flask and make up to the mark with distilled water.

iii) **Neutral 40% formalen solution:** Take 50 cm^3 of 40% formalin solution in a 250 cm^3 conical flask and add 8-10 drops of phenolphthalein indicator. To it add carefully from a burette a dilute solution of sodium hydroxide (0.1*M*), till the solution is just faintly pink.

iv) **Phenolpthalein indicator:** Dissolve 1.0 g of phenolphthalein in 100 cm^3 of ethanol and then dilute with 100 cm^3 of Water.

12.4 PROCEDURE

i) **Preparation of Standard solution of glycine:** Weigh accurately 2 g of glycine and transfer to a 250 cm^3 volumetric flask and make up to the mark with distilled water.

ii) **Titration with standard solution:** Take 25 cm^3 of standard glycine solution in a 250 cm^3 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 cm^3 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. Records the observations in Observation Table I.

iii) **Titration with unknown glycine solution:** Perform the titration as described above for 20 cm^3 unknown glycine solution and note the volume of sodium hydroxide used in this titration. Record the observations in Observation Table II

12.5 Observations

Mass of the weighing bottle	$= m_1$	=..... g
Mass of the bottle + glycine	$= m_2$	=..... g
Mass of the bottle	$= m_3$	=..... g
(after transferring the compound)		

Mass of glucose transferred $= m_2 - m_3 = m$ =..... 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	25			
2	25			
3	25			

Observation Table II
Unknown 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	25			
2	25			
3	25			

12.6 Calculations

The volume of sodium hydroxide solution used for 25 cm^3 of standard glycine solution $= V_1$ cm^3

The volume of sodium hydroxide solution used for 25 cm^3 of unknown glycine solution $= V_2$ cm^3

The amount of glycine in given solution $= \frac{m \times V_2}{V_1}$ = ... g

The strength of the unknown glycine solution $= \frac{4 \times m \times V_2}{V_1}$ = ...g dm^{-3}

$$= \frac{\text{Strength of the standard glycine solution} \times V_2}{V_1}$$

12.7 Result

The amount of glycine in the given solution = ... g

The strength of the unknown glycine solution = ... g dm^{-3}

EXPERIMENT 13
ESTIMATION OF FORMALDEHYDE

Structure

13.1 INTRODUCTION

Formaldehyde is usually available as an aqeous solution containing 33 to 37 percent by weight of formaldehyde. This aqeous solution is known as formalin. Here, we will describe one of the methods which has frequently to be applied for the estimation of formaldehyde in commercial formalin solution. In this method formaldehyde is oxidised quantitatively to formic acid by excess of iodine in alkaline solution. Then liberated iodine is titrated with standard sodium sulphate thiosolution.

The unreacted hypo-iodite is then acidified and the liberated iodine is titrated against sodium thiosulphate solution using starch as indicator. Further experimental details are given in next section.

Objectives

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

- determine the amount of formaldehyde as in given formaldehyde solution
- describe the oxidation reation of formaldehyde with sodium hypoiodite solution, and
- perform iodometric titration

13.2 PRINCIPLE

Formaldehyde may be estimated in solution by oxidising it to formic acid by means of a known quantity (in excess) of iodine dissolved in an excess of NaOH solution (hypoiodite solution). The formic acid thus formed is neutralised by the alkali present. The unreacted hypoiodite is then acidified with HCl and the liberated iodine is titrated with standard sodium thiosulphate solution using starch as indicator.

$$I_2 + 2NaOH \longrightarrow NaOI + NaI + H_2O$$

$$HCHO + NaOI + NaOH \longrightarrow HCOONa + NaI + H_2O$$

$$HCHO + I_2 + 3NaOH \longrightarrow HCOONa + 2NaCl + I_2 + H_2O$$

$$NaOI + NaI + 2HCl \longrightarrow 2NaCl + I_2 + H_2O$$

$$I_2 + 2Na_2S_2O_3 \longrightarrow Na_2S_4O_6 + 2NaI$$

13.3 REQUIREMENTS

Apparatus		Chemicals
Burette (50 cm^3)	- 1	Formalin solution
Pipette (25 cm^3)	- 1	Iodine
Vol. flasks (250 cm^3)	- 1	Sodium thiosulphate
Conical flask (250 cm^3)	- 1	Sodium hydroxide
Weighingbottle	-1	Conc.hydrochloric acid
Funnel (small)	- 1	Starch indicator
Wash-bottle for distilled water	- 1	Copper sulphate
Test-tube	- 1	
Burette stand	- 1	

Solutions Provided

i) **Iodine solution. 0.1 *M*:** Prepare iodine solution. (0.1*M*) of iodine by dissolving 3.17 g of it in 250 cm^3 volumetric flask in distilled water. Standardise it by titrating against standard sodium thiosulphate (0.1*M*) solution.

ii) **Sodium thiosulphale solution 0.1 *M* :** It is prepared by dissolving 6.25g sodium thiosulphate pentahydrate in distilled water in a 250 cm^3 volumatric flask.

iii) **Sodium hydroxide solution. 2*M*:** It is prepared by dissolving 40 gm sodium hydroxide in 500 cm^3 volumetic task distilled water.

iv) **Conc. hydrochloric acid. 2*M*:** It is prepared by taking 45 cm^3 of Conc.HCl and making up to the mark with in 250 cm^3 volumetric flask.

v) **Starch solution:** Make a paste of 1.0g of starch with a little water and pour the suspension with constant stirring into 100 cm^3 of boiling water.

13.4 PROCEDURE

i) **Formalin solution:** Weigh out accurately about 1.0 g of formalin solution, transfer it in a 250 cm^3 volumetric flask and make up to the mark with distilled water.

ii) **Titration with Iodine solution (Blank titration):** Pipette out 50 cm^3 of iodine solution in a 250 cm^3 conical flask. Titrate this solution with standard sodium thiosulphate solution. Sodium thiosulphate solution can be standarised by titrating against dichoromate solution. The procedurals details for the strandardisation is given in Experiment 9b. Repeat the titration to get atleast two concordant readings. Record the observation in Observation Table-I.

iii) **Titration with formaline Solution:** Pipette out 25 cm^3 of unknown formalin solution in a 250 cm^3 conical flask and add 50 cm^3 of 0.1*M* iodine solution. Solution develops a dark-brown colour. Now add 2*M* NaOH solution from the burette into the conical flask until the solution becomes pale yellow in colour. Shake the contents of the flask and allow to stand for 15 minutes. Acidify with 40 cm^3 of 2*M* hydrochloric acid to liberate the remaining iodine. Titrate this solution with sodium thiosulphate solution (0.1*M*) using starch as indicator.

Repeat the titration to get atleast two Concordant readings.

13.5 OBSERVATION

Observation Table I
Iodine Solution Vs. Sodium Thiosulphate Solution

Sl. No.	Volume of iodine Solution in cm^3	Burette reading		Volume of Sodium thiosulphate in cm^3 (Final–Initial)
		Initial	Final	
1	50			
2	50			
3	50			

Observation Table II
Formation and Iodine Solution Vs. Sodium Thiosulphate Solution

Sl. No.	Volume of Iodine solution added in cm^3	Burette reading		Volume of Sodium thiosulphate in cm^3 (Final–Initial)
		Initial	Final	
1	50			
2	50			
3	50			

13.6 CALCULATIONS

(a) Volume of 0.1*M* iodine solution added = 50 cm^3

(b) Volume of 0.1*M* sodium thiosulphate solution used in titration = *V* cm^3

Since V cm^3 0.1*M* sodium thiosulphate ≡ *V* cm^3 0.1*M* iodine

Hence, the volume of 0.1*M* iodine used = (50–*V*) cm^3

According to the equation of the reaction

$$HCHO + I_2 + 3NaOH \longrightarrow HCOONa + 2NaI + H_2O$$

From the above equation, it will be seen that 1 cm^3 of the *M*/10 iodine solution used in the oxidation is equivalent to 0.00150 g of formaldehyde.

Hence (50-*V*) cm^3 of 0.1*M* iodine solution = (50 – *V*)×0.00150 g of HCHO

$2\ dm^3\ 0.1\ M\ I_2 \equiv 1 dm^3$ of 0.1 *M* HCHO

$1\ dm^3$ of 0.1 $MI_2 \equiv 30/2 \times 10$ g *H*CHO

$1\ cm^3$ of 0.1 $MI_2 \equiv .00150$ g *H*CHO

25 cm^3 of supplied solution contains = (50 – *V*)×0.00150 g of HCHO

Percentage of formaldehyde in the given solution = $\frac{(50 - V)\times 0.00150\times 100}{25}$ =... %

13.7 Result

Percentage of formaldehyde in the solution =..... %

In next experiment we will describe the analysis of the oils and fats. This experiment will tell us how we can use organic quantitative methods to determine different parameters such as saponification value, iodine value and acid value of given oils or fat.

EXPERIMENT 14
ANALYSIS OF OILS AND FATS:

Structure

14.1 INTRODUCTION

Oils and fats are triglycerides with three long chains of fatty acid group randomly esterified with glycerol. The difference between oils and fats is that, oil are liquid whereas fats are solid at ordinary temperature.

Fats and oils mainly come from plant seed. In animals they are present under the skin, in tissues and muscles. Some of the fatty acids like linoleic, linolenic and arachidonic acids are very essential for our body. Fats and oils are widely distributed in food and are of great nutritional value. They are also used in manufacture of soaps, detergents, glycerine, candles, printing ink etc.

The industrial value of a particular oil or fat depends upon its physical and chemical characteristics, e.g. melting point, specific gravity, refracting index, viscosity, saponification value, iodine value, acid value, acetyl value, etc. In this experiment we will determine some of these parameters, such as saponification value, iodine value and acid value.

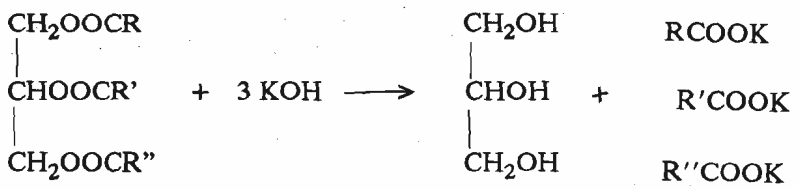
Objectives

After studying and performing this experiment you should be able to

- define the term saponification value, iodine value and acid value, and
- determine saponification value, iodine value and acid value.

14.2 DETERMINATION OF SAPONIFICATION VALUE

Esters are hydrolyzed either by aqueous base or by aqueous acid to yield component acid and alcohol fragments. Hydrolysis of an ester in alkaline medium is called saponification.



Saponification number or saponification value is an arbitrary unit that is defined as "number of mg of potassium hydroxide required to saponify 1gm of oil or fat" i.e. to neutralise completely the fatty acids resulting from complete hydrolysis of 1gm fat or oil.

The saponification value gives an idea about the molecular weight of fat or oil. The smaller the saponification value, the higher the molecular weight. As the average molecular weight of oil or fat depends on the average length of carbon chain of the fatty acid components, the saponification value also gives an indication of the average length of the carbon chain in the oils or fats. The saponification value for each oil has its own characteristic value.

14.2.1 Principle

Boiling of the sample under reflux condenser with ethanolic KOH solution, and titration of excess potassium hydroxide with standard HCl in presence of an indicator.

14.2.2 Requirements

Apparatus

Conical flask - 250 cm^3
Reflux condenser
Pipette
Burette - 50 cm^3
Water bath

Chemicals

Standard HCl (*M*/2)
Alcohol
Potassium hydroxide
Phenolphthalein

Solution Provided

2.5 *M* KOH Solution: It can be prepared by dissolving 24.2g KOH in 100 cm^3 water.

Procedure

1. Weigh accurately about 1g of oil in 250 cm^3 conical flask,
2. Dissolve the ester in 25 cm^3 alcohol and then add 25 cm^3 of 2.5*M* KOH solution with the help of a pipette,
3. Attach a reflux water condenser to the flask and add some boiling chips,
4. Heat the flask in water bath for about 1 hour with occasional shaking,
5. After 1 hour, stop heating. Add to the hot solution 0.5 to 1.0 cm^3 of phenolphthalein,
6. Titrate the excess alkali with standard *M*/2 HCl until the colour of the indicator changes. Record the volume of HCl used in Observation Table-II.
7. Carry out determination with the same prepared sample again to get at least two concordant readings.
8. Carry out a blank test upon the same quantity of KOH solution at the same time under the same conditions. Record the volume of HCl used in Observation Table-I.

14.3.3 Observations

Mass of the weighing bottle,	m_1 =	.... g
Mass of the bottle + oil,	m_2 =	.... g
Mass of the bottle, (after transferring the oil)	m_3 =	.... g

Mass of oil transferred, $m_2 - m_3$ = m= .... g

Observation Table-I
(Blank Experiment)
KOH Solution vs. HCl

Sl. No.	Volume of KOH Solution in cm^3	Burette Reading Initial	Burette Reading Final	Volume of HCl solution used in cm^3 (Final–Initial)
1.				
2.				
3.				

Observation Table-II
(Original Experiment)
Oil Sample + KOH Solution vs. HCl

Sl. No.	Volume of oil Sample + KOH Solution in cm^3	Burette Reading Initial	Burette Reading Final	Volume of HCl solution used in cm^3 (Final–Initial)
1.				
2.				
3.				

14.3 .4 Calalation and

Result

The saponification value (S.V.) is given by the formula:

$$\text{S.V.} = \frac{56.1 \times M_m \times (V_0 - V_1)}{m}$$

where: V_0 = Volume of HCl used in the blank titration cm^3

V_1 = Volume of HCl used in the original titration cm^3

M_m = Molarity of HCl

m = mass of the oil in g.

14.3 DETERMINATION OF IODINE VALUE

Iodine value or iodine number of fats or oil is a measure of its degree of unsaturation and gives an idea of its drying character. Iodine value depends on the number of double bond present in the molecule. Low iodine value means that the carbon chain of triglyceride contains very few carbon-carbon double bond, while a higher iodine number indicates a large number of double bonds present in the molevle. If the triglyceride do not contain any double bond then the iodine value will be zero.

Iodine value can be defined as "the number of grams of halogen absorbed by 100 grams of the oils fat, and expressed as the weight of iodine.

14.3.1 Principle

A know weight of oil is treated with ICI solution in CCl_4 or $CHCl_3$ and the amount of ICl absorbed is determined. Iodine number is thus determined. In this process following reaction occurs:

$$>C=C< + ICl \longrightarrow >\underset{\displaystyle I}{\overset{\displaystyle |}{C}}-\underset{\displaystyle Cl}{\overset{\displaystyle |}{C}}<$$

After the completion of the reaction KI solution is added which is oxidised to I_2 by unreacted ICl

$$ICl + KI \longrightarrow I_2 + KCl$$
(unused)

The liberated iodine is titrated with standard NaS_2O_3 solution using starch solution as indicator.

$$I_2 + 2Na_2S_2O_3 \longrightarrow 2NaI + Na_2S_2O_6$$

14.3.2 Requirements

Apparatus

The apparatus must be clean and perfectly dry.

1. Conical flask (250 cm^3) 1
2. Burette (50 cm^3) 1
3. Pipette (25 cm^3) 1

Chemicals

1. Glacial acetic acid
2. Carbon tetrachloride
3. Iodine trichloride
4. Iodine, pure, resublimed
5. Potassium iodide,
6. Sodium thiosulphate,
7. Starch solution

Solution Provided

Alternatively Wijs solution may be prepared by dissolving separately 7.9g of iodine trichloride and 8.7g of pure iodine in glacial acetic acid, mixing the two solution and diluting them to one dm^3 with glacial acetic acid.

Sodium Thiosulphate (*M*/10): It can be prepared by dissolving 24.8g $Na_25_20_3$ in water in a 1dm^3 volumetric flask.

Potassium iodide: It can be prepared by dissolving 100 g potassium iodide in 1 dm^3 water.

Wijs Solution (ICl): This can be prepared by dissolving 9 gm of iodine trichloride in a mixture of 700 cm^3 glacial acetic acid (pure) and 300 cm^3 carbon tetrachloride.

14.3.3 Procedure

The amount of oil to be taken varies according to its expected iodine value and is given in the following table:

Iodine Value expected	Weight to be taken (g)
0-5	3.0
5-20	1.0
21-50	0.40
51-100	0.20
101-150	0.15
151-200	0.10

1. Dissolve the appropriate quantity of oil, in a 250 cm^3 conicalflask in 15 cm^3carbontetrachloride.
2. Add 25 cm^3 of Wijs solution, from the burette, to the mixture.
3. Close the flask, shake gently and allow it to stand in dark at about 20°C.
4. For oils having iodine value below 150, leave the flask in dark for 1 hour; for those with iodine value above 150 leave the flask for 2 hours.
5. After that add 25 cm^3 of potassium iodide solution and 150 cm^3 of water.
6. Titrate the liberated iodine with standard M/10 sodium thiosulphate solution, using starch solution as indicator, continue the titration until the blue colour just disappears. Record the value of $Na_2S_2O_3$ used in Observation Table-IV
7. Carry out experiment again to get at least two correspondent readings.
8. Carry out a blank test with out oil under the same conditions. Record the volume of $Na_2S_2O_3$ used in Observation Table-III

If 15cm^3 is insufficient to dissolve the oil, large amount of oil may be added. This should be mentioned in the report of the analysis.

14.3.4 Observation:

Mass of the weighing bottle, $=m_1$ = ... g

Mass of the bottle + oil, $=m_2$ = ... g

Mass of the bottle, $=m_3$ = ... g

(after transferring the oil)

Mass of the oil transferred, $=m_2 - m_3 = m$ = ... g

Observation Table- III
(Blank Experiment)'
ICl solution vs. M/10 Sodium Thiosulphate Solution

Sl.No. No.	Volume of ICl solution in cm^3	Burette Reading Initial	Final	Volume of Sodium thiosulphate solution used in cm^3 (Final–initial)
1.				
2.				
3.				

Observation Table-IV
(Original Experiment)
Oil Sample + ICl Solution Vs. M/10 Sodium Thiosulphate Solution

Sl.No.	Volume of Oil Sample + ICl solution in cm^3	Burette Reading Initial	Final	Volume of Sodium thiosulphate used in cm^3 (Final–initial)
1.				
2.				
3.				

14.3.5 Calculations and Result

The iodine value (I.V.) is given by the formula:

$$\text{I.V.} = \frac{12.69 \times M \times (V_0 - V_1)}{m}$$

where: V_0 = Volume of sodium thiosulphate used in blank titration

V_1 = Volume of sodium thiosulphate used in original titration

M = Molarity of sodium thiosulphate

m = mass of the oil.

14.4 Determination of Acid Value

rancidity : offensively strong taste or smel when the oil or fat is going bad

Acid value of oils or fats indicates the amount of free fatty acid present in it. The acid value can give the extent of rancidity in a sample. The acid value can be defined as "the number of mg of potassium hydroxide required to neutralise the free fatty acid present in 1 gm of oil or fat.

This standard is applicable to animal and vegetable oils and fats. It is not applicable to waxes.

14.4.1 Principle

Solution of a known quantity of the fat to be analysed in a mixture of ethanol and diethylether, followed by titration of the free fatty acids present with an ethanolic solution of KOH.

14.4.2 Requirements

Apparatus			**Chemical**
1.	Conical flask (250 cm^3)	2	NaOH
2.	Burette (25 cm^3)	1	Ethanol
3.	Pipitte (25 cm^3)	1	Phenolphthalein
			KOH
			Diethyl ether.

Solution Provided

Diethyl ether may be substituted by toluene

1. **Solvent:** It can be prepared by mixing equal volume of 95% ethanol and diethyl ether, then it is neutralized shortly before use, with 0.1M NaOH in presence of 0.3cm^3 of phenolphthalein solution per 100 cm^3 of the mixture.

2. **Potassium hydroxide: 0.1M.** It can be prepared by dissolving 5.4 g KOH in water in 1 dm^3 volumetric flask. It can be standardized by 0.2 M standard oxalic acid solution.

3. **Phenolphthalein indicator:** 1% solution in 95% ethanol.

14.4.3 Procedure

The weight of the oil are chosen in accordance with the following table.

Expected A.V	Approx. amount of oil in g
1	20
1-4	10
4-15	2.5
15-75	0.5
75	0.1

1. Weigh accurately appropriate quantity of oil in a 250 cm^3 conical flask.
2. Dissolve the oil in about 150 cm^3 of the solvent mixture. (mixture of ethanol and ether). Shake the mixture to dissolve the oil.

3. Add few drops of phenolphthalein.
4. Titrate the mixture, keep shaking, with the standard solution of KOH until pink colour disappears .
5. Record the volume of KOH used in Observation Table-V. Repeat the experiment to get at least two correspondent readings.

If the solution becomes cloudy during titraction, add further quantity of solvent mixture

Note: If the quantity of 0.1M KOH solution required exceeds 20 cm^3, 0.5M solution should be used.

14.4.4 Observations

Mass of the weighing bottle, $= m_1$ = ... g

Mass of the bottle + oil, $= m_2$ = ... g

Mass of the bottle, $= m_3$ = ... g

(after transferring the oil)

Mass of the oil transferred, $= m_2 - m_3$ = ... g

Observation Table-V
Oil Sample solution vs. Standard KOH solution

Sl.No.	Burette Reading		Volume of KOH solution used in cm^3 (Final–Initial)
	Initial	Final	
1.			
2.			
3.			

14.4.5 Calculations and

Result

The acid value (A.V.) is given by the formula:

$$\text{A.V.} = 56.1 \times M \times \times \frac{V}{m}$$

where: V = Volume of the KOH solution used

M = Molarity of the KOH solution

m = mass of the oil.

EXPERIMENT 15
ESTIMATION OF MAGNESIUM AND CALCIUM IONS IN A MIXTURE BY COMPLEXOMETRY

Structure

15.1 INTRODUCTION

In Chemistry Lab-I and Chemistry Lab-II courses, you performed titrimetric or gravimetric estimations of only single cation present in any substance. In this experiment and Experiments 15 and 16, you will perform titrimetric or gravimetric estimations of two cations together. For example, you will determine i) magnesium and calcium by complexometry; ii) copper and zinc, and iii) copper and nickel by gravimetry. Now we will concentrate on complexometric titration method.

Numerous methods are available for titrimetric determination of various cations by titrations of their salts with certain organic reagents called complexones. These complexones are imino-polycarboxylic acids, having excellent complex forming ability with a number of cations. The simplest of the complexones is iminodiacetic acid, $HN(CH_2COOH)_2$. Other complexones can be assumed to be higher derivatives of this family. The most important member of this family of reagents is ethylenediaminetetraacetic acid, abbreviated as EDTA. The structure of EDTA is shown in Fig. 15.1.

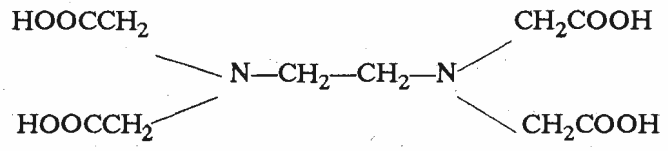


Fig. 15.1: The structure of EDTA

EDTA can form complexes with a number of cations like alkaline earth metals and many non-ferrous metal ions like Cu^{2+}, Zn^{2+}, Pb^{2+}, Co^{2+}, Mn^{2+}, Bi^{3+}, Zr^{4+} and Hf^{4+}, etc.

EDTA is only slightly soluble in water. However, its disodium salt is freely soluble in water. Dihydrate of the disodium salt is available commercially in a state of high purity under the brand names 'Versen' or 'Trilon-B'. It can be used as a primary standard. EDTA, generally, forms 1:1 complexes with metal ions. In reactions, EDTA and its disodium salt are represented as H_4Y and Na_2H_2Y, respectively. Reaction of the disodium salt with a bivadent cation can be written as follows:

$$Na_2H_2Y \longrightarrow 2Na^+ + H_2Y^{2-}$$

$$H_2Y^{2-} + M^{2+} \longrightarrow MY^{2-} + 2H^+$$

It is apparent from the above equation that there is always a competition in solution between the metal ions and the hydrogen ions in seeking the negative sites on EDTA. The equilibrium condition is determined by the strength of the bond between the metal ion and the ligand, and the relative concentrations of metal ions versus hydrogen ions. In other words, we can say that the stability of the metal-EDTA complex will be governed by the hydrogen ion concentration or pH of the solution. Minimum pH values for the stability of EDTA complexes of some selected metal ions are listed in Table 15.1

Table 15.1: Stability with respect to pH of some Metal EDTA complexes

Sl.No.	Metal ion	Minimum pH at which complex is stable
1.	Bi^{3+}, Zr^{4+}, Hf^{4+}, Th^{4+}	1 - 3
2.	Pb^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}, Sb^{2+}, Fe^{2+}	4 - 6
3.	Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}	8 - 10

You can see from the Table that in general, EDTA complexes with alkaline earth metal ions are stable in alkaline solution, whilst complexes with tri- and tetra-valent metal ions are stable in strongly acidic solutions.

EDTA is a multidentate ligand as it can donate six paris of electrons - two pairs from the two nitrogen atoms and four pairs from the four terminal oxygens of the $-COO^-$ groups. Such multidentate ligands prefer to form complexes having ring type structures. As you know, these complexes are called chelates and such ring forming ligands are called chelating agents.

The structure of a chelate of a divalent metal ion with EDTA is shown in Fig. 15.2.

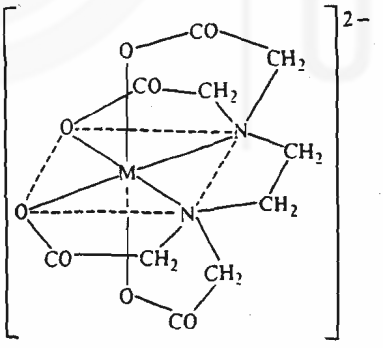


Fig. 15.2: The structure of a chelate of M^{2+} and EDTA

Objectives

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

- estimate Ca^{2+} and Mg^{2+} together by complexometry, and
- describe complexones and perform complexometric titration

15.2 PRINCIPLE

In complexometric determination of magnesium and calcium ions in their mixture, EDTA is used as a titrant and Solochrome Black (Eriochrome Black T) as an indicator. When indicator solution, which is blue in colour, is added to the solution containing magnesium and calcium ions, wine red coloured metal ion-indicator

complexes of varying stability are formed. The magnesium-indicator complex is more stable than the calcium indicator complex but less stable than the magnesium-EDTA complex which in turn is less stable than the clacium-EDTA complex. Consequently, when EDTA solution is added, it reacts first with the free calcium ions, then with the free magnesium ions, then with the calcium indicator complex and finally with the magnesium-indicator complex. Since the magnesium-indicator complex is wine-red in colour and the free indicator is blue between pH 7 and 11, the colour of the solution changes from wine-red to blue at the end point.

In this experiment, we titrate one portion of the test solution containing both magnesium and calcium ions with EDTA using Solochrome Black indicator at pH 10 and the volume consumed is noted. This gives the volume of EDTA required for the titration of both magnesium and calcium ions. Then another equal portion of test solution is taken, but this time the medium is kept strongly alkaline. In strong alkaline medium, magnesium ions are precipitated and the calcium ions are left free in the solution. This solution is then titrated with EDTA for calcium ions only using Murexide as indicator. The volume of EDTA consumed is the volume required for titration of calcium ions only. By substracting the volume of EDTA consumed by calcium ions from the volume of EDTA required for both magnesium and calcium ions, we get the volume required for magnesium ions.

As EDTA is a primary standard, its molarity is known. Then using the molarity equation, $M_1V_1 = M_2V_2$, the molarity of magnesium and calcium ions can be calculated.

15.3 REQUIREMENTS

You will need the following apparatus, chemicals and solutions for this experiment.

Apparatus		**Chemicals**
Beaker 250 cm^3	1 No.	Ammonia liquor
Burette 50 cm^3	1 No.	Ammonium chloride
Burette stand	1 No.	Calcium chloride
Conical flask 250 cm^3	1 No.	Disodium salt of EDTA
Funnel	1 No.	Magnesium sulphate
Pipette 20/25 cm^3	1 No.	Murexide indicator
Pipette graduated 10 cm^3	1 No.	Sodium hydroxide
Volumetric flask 250 cm^3	1 No.	Solochrome Black indicator
Wash bottle	1 No.	
Weighing bottle	1 No.	

Solutions provided :

1. **Test solution :** It can be prepared by dissolving accurately 2–3 g of calcium chloride and 1–2 g of $MgSO_4$ into minimum quantity of dil. HCl and making up the volume to 250 cm^3 with distilled water.

2. **$NH_3 - NH_4Cl$ buffer solution of pH 10 :** This can be prepared by dissolving 64g of NH_4Cl in distilled water, adding 570 cm^3 of ammonia solution (sp.gr. 0.88) and diluting to 1dm^3 with disttled water.

3. **Solochrone Black indicator (0.5% mass /volume) :** 0.50 g indicator is weighted and dissolved in 100 cm^3 ethanol.

4. **Mureride indicator :** It can be used as solid, in 0.05g quantity in each titration. The indicator solution may be prepared by suspending 0.5g of the powered solid in

water, shaking thoroughly, and allowing undissolved protion to settle. The saturated supernant liquid is used for titrations. Every day the old supernatant liquid decanted and the residue treated with water as before to provide a fresh solution of indicator. 3–4 drops of this solution are used for each litration.

5. **0.1 *M* NaOH solution :** Dissolve 4 g of NaOH in 1 dm^3 of distilled water.

15.4 PROCEDURE

The experimental procedure involves the following steps:

1) Preparation of standard 0.1*M* EDTA solution: As said earlier, EDTA is available as a dihydrate of its disodium salt ($Na_2H_2Y.2H_2O$). Take already dried disodium salt of EDTA from your counsellor. Take rough mass of a glass weighing bottle, transfer about 9.5 g of the salt to the weighing bottle and weigh accurately. 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 weighing bottle after transferring the salt. The difference between the two masses gives the actual mass of the salt taken. Record these values in your observation note book for calculating the exact concentration of the solution. Now dissolve the salt in deionised or distilled water. Make up to the mark with distilled water and shake thoroughly to make a homogeneous solution.

2) Titration of the test solution

i) Fix a clean burette in a burette stand.

ii) Fill the burette with the EDTA salt solution after rinsing it with this solution and mount the burette on a stand. Note the reading in the burette and record it in the Observation Table I under the column 'Initial reading'.

In this titration colour change develops a little late, hence, titration should be done slowly. If necessary, add 2-3 drops more of indicator at the final stage of titration. This will provide necessary contrast in colour.

iii) Pipette out 25 cm^3 of test solution in a 250 cm^3 conical flask. Add 10 cm^3 of 0.1 *M* NaOH solution and 3-4 drops of Murexide indicator solution. Dilute it to 50 cm^3 with distilled water. Now titrate with EDTA solution until the colour changes from red to purple. Repeat the titration 3-4 times to obtain concordant values. Record the values in Observation Table I under the column 'Final Reading'. This gives volume (V_1) of EDTA required for calcium ions only.

iv) Pipette out 25 cm^3 of another portion of the test solution containing both magnesium and calcium ions, in a 250 cm^3 conical flask. Add 5 cm^3 of buffer solution (pH = 10) and dilute it to 50 cm^3 with distilled water. Ensure that the smell of ammonia persists. If necessary add 2-3 drops of liquor ammonia. Add Solochrome Black indicator (3-4 drops) and warm up to 50-60°C. Now, titrate with EDTA solution till the wine red colour of the solution changes to bluish. Note the final reading in the burette and record it in the Observation Table II under the column 'Final reading'. Repeat the titration 3-4 times till concordant values are obtained. This gives the volume (V_2) of EDTA required for both calcium and magnesium ions.

Solution should be warmed to 50–60°C, but under no circumstances it should be boiled.

15.5 OBSERVATIONS

Approximate mass of the weighing bottle = m_1 =..... g

Mass of the weighing bottle + EDTA salt = m_2 =..... g
(before transferring the salt)

Mass of the weighing bottle = m_3 =..... g
(after transferring the salt)

Observation Table I

Titration of the test solution with EDTA using Murexide indicator

Sl. No.	Volume of test solution in cm^3	Burette reading Initial	Final	Volume of EDTA salt, V_1 in cm^3 (Final–Initial)
1.	25			
2.	25			
3.	25			
4.	25			

Observation Table II

Titration of the test solution with EDTA using Solochrome Black indicator

Sl. No.	Volume of test solution in cm^3	Burette reading Initial	Final	Volume of EDTA salt, V_2 in cm^3 (Final–Initial)
1.	25			
2.	25			
3.	25			
4.	25			

15.6 CALCULATIONS

Molarity of EDTA salt solution

Mass of EDTA salt transferred (m) = $m_2 - m_3$= ..... g

Molar mass (M_m) of sodium salt of EDTA = 372.3 g mol^{-1}

Volume of EDTA salt solution prepared = 250 cm^3

Molarity of EDTA salt solution = $M_1 = \frac{m \times 1000}{M_m \times 250}$ mol dm^{-3}

$= \frac{m \times 4}{372.31}$ mol dm^{-3}

Concentration of Calcium ions in solution

Volume of calcium ion solution = V_2 = 25 cm^3

Molarity of Calcium ion solution = M_2 = ?

Volume of EDTA salt solution required for calcium ions = V_1 cm^3 (From Table I)

Molarity of EDTA salt solution = $M_1 = \frac{m \times 4}{372.31}$

Now, using the molarity equation, $M_1V_1 = M_2V_2$, we get,

$$M_2 = \frac{M_1V_1}{V_2}$$

$$= \frac{m \times 4 \times V_1}{372.31 \times 25} \text{ g } dm^{-3}$$

Hence, concentration of calcium ions = molarity × molar mass of Ca^{2+}

$$= \frac{m \times 4 \times V_1}{372.31 \times 25} \text{ mol } dm^{-3} \times (40.08 \text{ g } mol^{-1})$$

$$= \frac{m \times 4 \times V_1 \times 40.08}{372.31 \times 25} \text{ g } dm^{-3}$$

Concentration of Magensium ions in solution

Volume of magnesium ion solution $= V_3 = 25\ cm^3$

Molarity of magnesium ion solution $= M_3 = ?$

Volume of EDTA salt solution required for magnesium ions $= (V_2 - V_1)\ cm^3$

Molarity of EDTA salt solution $= M_1 = \frac{m \times 4}{372.31}$

Now, using the molarity equation, we get,

$$M_1 (V_2 - V_1) = M_3 V_3$$

or $$M_3 = \frac{M_1 (V_2 - V_1)}{372.31 \times 25}$$

$$= \frac{m \times 4 \times (V_2 - V_1)}{372.31 \times 25} \text{ mol dm}^{-3}$$

Hence, concentration of magnesium ions = molarity × moar mass of Mg^{2+}

$$= \frac{m \times 4 \times (V_2 - V_1)}{372.31 \times 25} \text{ mol dm}^{-3} \times (24.32 \text{ g mol}^{-1})$$

$$= \frac{m \times 4 \times (V_2 - V_1) \times 24.32}{372.31 \times 25} \text{ g dm}^{-3}$$

15.7 RESULT

You can report your result in the following form:

1. Calcium content in the solution =..... g dm^{-3}
2. Magnesium content in the solution =..... g dm^{-3}

EXPERIMENT 16
ESTIMATION OF COPPER AND ZINC IN A MIXTURE BY GRAVIMETRY

Structure

16.1 INTRODUCTION

In the estimation of copper and zinc in a given mixture, we first estimate copper as copper(I) thiocyanate (CuCNS) and then in the filtrate, we estimate zinc as zinc ammonium phosphate ($ZnNH_4PO_4$). The test solution, if not provided by the counsellor, can be prepared by dissolving accurately 5-6 grams of copper sulphate and 2-3 grams of zinc chloride in a minimum quantity of dilute HCl and then making up the volume to 250 cm^3 in a standard volumetric flask. 50 cm^3 of this solution can be taken for estimation.

Objectives

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

- estimate Cu^{2+} and Zn^{2+} together by gravimetry.

16.2 PRINCIPLE

The test solution, containing copper and zinc, is first treated with sulphurous acid or ammonium hydrogen sulphite to reduce Cu(II) to Cu(I). Ammonium thiocyanate solution is then added to precipitate copper as copper(I) thiocyanate. The reactions that take place are shown below.

$$2Cu^{2+}(aq)+HSO_3^-(aq)+H_2O(l) \longrightarrow 2Cu^+(aq)+HSO_4^-(aq)+2H^+(aq)$$

$$Cu^+(aq)+SCN^-(aq) \longrightarrow CuSCN(s)$$

After filtering the precipitate, diammonium hydrogen phosphate solution is added to the filtrate to precipitate zinc as zinc ammonium phosphate:

$$ZnCl_2(aq) + (NH_4)_2HPO_4(aq) \longrightarrow ZnNH_4PO_4(s) + HCl(aq)$$

The precipitate is filtered, washed, dried and weighed. By knowing the mass of CuSCN and $ZnNH_4PO_4$ formed, we can calculate the concentrations of copper sulphate and zinc chloride in the solution.

The names and atomic/molar masses of species involved in this experiment are given below.

Name	Formula/Symbol	Molar/Atomic mass
Copper(II) sulphate	$CuSO_4.5H_2O$	249.50
Copper(I) thiocyanate	CuSCN	121.62
Zinc ammonium phosphate	$ZnNH_4PO_4$	178.34
Zinc chloride	$ZnCl_2$	136.27
Zinc	Zn	65.37
Copper	Cu	63.54

16.3 REQUIREMENTS

You will need the following apparatus, chemicals and solutions for this experiment

Apparatus		Chemicals
Beaker 500 cm^3	2 Nos	10% Ammonium thiocyanate
Beaker 250 cm^3	1 No.	Aqueous ammonia
Bunsen burner	1 No.	10% Diammonium hydrogen phosphate
Desiccator	1 No.	Conc. Hydrochloric acid
Filtration apparatus	1No.	Methyl red indicator
Flask conical 250 cm^3	1 No.	Conc. Nitric acid
Glass rod	1 No.	Ammonium hydrogen sulphite solution
Pair of tongs	1 No.	Test Solution
Rubber police-man	1 No.	
Sintered crucible (G-4)	1 No.	
Tripod stand	1 No.	
Wash bottle	1 No.	
Watch glass	1 No.	
Water bath	1 No.	
Wire guaze	1 No.	
Weighing bottle	1 No.	
Weight box	1 No.	

16.4 PROCEDURE

The experimental procedure involves the following steps:

(A) Estimation of copper

I. Pipette out 50 cm^3 of test solution either assigned by your counsellor or prepared by you in a 500 cm^3 beaker. Add into it 5 cm^3 dilute HCl and 25 cm^3 ammonium hydrogen sulphite solution and ensure that smell of SO_2 prevails.

II. Boil gently on a water bath. Remove the flame and add slowly 50 cm^3 of 10% NH_4SCN solution. Keep stirring the precipitate intermittently with a glass rod. Allow the solution, containing the precipitate, to rest for 30-60 minutes.

III. Weigh an empty and cleaned sintered glass crucible of porosity G- 4 and note the mass. Filter the precipitate, obtained in step II, through this crucible first by draining off the supernatant liquid and then the precipitate with minimum

quantity of liquid. By this procedure the precipitate is kept away from clogging the pores of the crucible.

IV. Wash the precipitate with 2-3% NH_4SCN solution. Wait and check the filtrate for any precipitate formed. If any precipitate appears, filter it again. Finally wash the precipitate several times with 20% ethanol until the precipitate is free from SCN^- ions. Preserve the filtrate for estimation of zinc.

V. Now heat the sintered glass crucible at a temperature of 100- 120°C in an hot air oven for at least an hour.

VI. Cool the crucible in a desiccator and then weigh. Repeat the process of heating, cooling and weighing until a constant mass of the crucible with precipitate is obtained.

(B) Estimation of zinc

I. Evaporate on a water bath, the filtrate obtained in step IV in the estimation of copper. Reduce the volume of the solution to nearly 100 cm^3 by evaporation. Now add 20 cm^3 concentrated nitric acid and 15 cm^3 concentrated HCl and again heat to dryness on a water bath in a fume hood.

II. To the residue, obtained in step I, add 100 cm^3 of distilled water and dissolve the contents by shaking. Add 2-3 drops of methyl red indicator and then add 10% aqueous ammonia solution till smell of ammonia prevails and the colour of the methyl red changes to yellow.

III. To the solution obtained in step II, add slowly 10% diammonium hydrogen phosphate with adequate stirring with a glass rod. Digest the precipitate on a water bath atleast for 30 minutes.

IV. Filter the precipitate, obtained in step III, through a previously weighed sintered crucible of porosity G-4. Drain out the supernatant liquid first and then the precipitate with minimum quantity of liquid.

V. Wash the precipitate with 2-3% diammonium hydrogen phosphate (DAHP) solution. Finally wash the precipitate with 50% alcohol to remove the excess of DAHP. Check if any phosphate ions are present in the washings.

VI. Heat the crucible and the precipitate at temperature range 100- 120°C in an hot air oven. Cool the crucible in a desiccator and then weigh it. Repeat heating, cooling and weighing till a constant mass of the crucible with precipitate is obtained.

16.5 OBSERVATIONS

i)	1st mass of empty crucible	=	..... g
ii)	2nd mass of empty crucible	=	..... g.
iii)	1st mass of crucible + CuSCN	=	..... g
iv)	2nd mass of crucible + CuSCN	=	..... g
v)	1st mass of empty crucible	=	..... g
vi)	2nd mass of empty crucible	=	..... g
vii)	1st mass of crucible + $ZnNH_4PO_4$	=	..... g
viii)	2nd mass of crucible + $ZnNH_4PO_4$	=	..... g

16.6 CALCULATIONS

Calculations for copper

Mass of CuSCN obtained = iv) – ii)= ..... g = w g

From stoichiometry we know,

$CuSO_4.5H_2O \equiv Cu^{2+} \equiv CuSCN$

249.5 g ≡ 63.54 g ≡ 121.62 g

Hence, w g of copper(I) thiocyanate $\equiv \frac{249.5 \times w}{121.62}$ g of copper(II) sulphate.

This much copper(II) sulphate is present in 50 cm^3 of test solution. Hence, concentration of copper(II) sulphate in test solution

$$= \frac{249.5 \times w}{121.62} \times \frac{1000}{50} \text{ g dm}^{-3}$$

$$\text{Concentration of } CuSO_4.5H_2O = \frac{249.5 \times 1000 \times \text{ mass of CuSCN}}{121.62 \times \text{ volume of test solution}} \text{ g dm}^{-3}$$

Calculations for zinc

Mass of $ZnNH_4PO_4$ obtained = viii) – vi) =..... g = w' g

From stoichiometry we know

$ZnCl_2 \equiv Zn^{2+} \equiv ZnNH_4PO_4$

136.37 g ≡ 65.37 g ≡ 178.34 g

Hence, w' g of $ZnNH_4PO_4 \equiv \frac{136.37 \times w'}{178.34}$ g of $ZnCl_2$

This much $ZnCl_2$ is present in 50 cm^3 of test solution.

Hence, concentration of $ZnCl_2$ in test solution

$$= \frac{136.37 \times w' \times 1000}{178.34 \times 50} \text{ g dm}^{-3}$$

$$\text{Concentration of } ZnCl_2 = \frac{136.37 \times 1000 \times \text{ mass of } ZnNH_4PO_4}{178.34 \times \text{ Volume of test solution}} \text{ g dm}^{-3}$$

16.7 RESULT

You can report your result in the following manner:

Concentration of copper(II) sulphate in the test solution = ... g dm^{-3}

Concentration of zinc chloride in the test solution = ... g dm^{-3}

EXPERIMENT 17
ESTIMATION OF COPPER AND NICKEL IN A MIXTURE BY GRAVIMETRY

Structure

17.1 INTRODUCTION

In the previous experiment we have estimated copper and zinc gravimetrically. In this experiment we are again going to use gravimetric method for estimation of copper and nickel together in a mixture.

Objectives

After studying and performing this experiment you should be able to:

- estimate copper and nickel together by gravimetry.

17.2 PRINCIPLE

In the estimation of copper and nickel in a given mixture, we first estimate copper as cuprous thiocyanate (CuSCN) and then in the filtrate we estimate nickel as nickel dimethylglyoximate, $Ni(HDMG)_2$. The test solution, if not assigned by your counsellor, can be prepared by dissolving accurately 5-6 grams of copper(II) sulphate and 2-3 grams of nickel(II) sulphate in a minimum quantity of dilute HCl and then making up the volume to 250 cm^3 in a standard volumetric flask. 50 cm^3 of this solution can be taken for estimation.

The test solution, containing copper and nickel, is treated with ammonium thiocyanate solution whereby only copper gets precipitated as copper(I) thiocyanate leaving behind nickel in the solution. Before precipitating copper as CuSCN, we add sulphurus acid in test solution so as to keep any, Cu(II) reduced to Cu(I) state. The reactions that take place are as follows·

$$2\,Cu^{2+}(aq) + HSO_3^-(aq) + H_2O(l) \longrightarrow 2Cu^+(aq) + HSO_4^-(aq) \longrightarrow H^+(aq)$$

$$2Cu^+(aq) + SCN^-(aq) \rightarrow 2CuSCN(s)$$

$$2Cu^{2+}(aq) + 2\,SCN^-(aq) + HSO_3^-(aq) + H_2O(l) \longrightarrow$$

$$2\,CuSCN(s) + HSO_4^-(aq) + 2H^+(aq)$$

After filtering CuSCN, in the filtrate which is free from copper, we add a solution of dimethylglyoxime to precipitate nickel as nickel dimethylglyoximate. This complex is of scarlet red colour. The chemical reaction that takes place is as under:

$$Ni^{2+}(aq) + 2H_2DMG(aq) \rightarrow Ni(HDMG)_2(s) + 2H^+(aq)$$

The structure of nickel dimethylglyoximate is shown in Fig. 16.1.

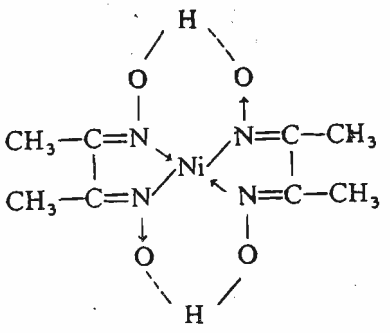


Fig. 16.1: The Structure of $Ni(HDMG)_2$

The names and atomic/molar masses of the species involved in this experiment are given below.

Name	Formula/Symbol	Molar/Atomic mass
Copper(II) sulphate	$CuSO_4.5H_2O$	249.50
Copper(I) thiocyanate	CuSCN	121.62
Copper	Cu	63.54
Nickel(II) sulphate	$NiSO_4$	154.69
Nickel(II) dimethylglyoximate	$Ni(HDMG)_2$	288.91
Nickel	Ni	58.69

17.3 REQUIREMENTS

You will need the following apparatus, chemicals and solutions for this experiment.

Apparatus		Chemicals
Beaker 500 cm^3	2 Nos	10% Ammonium thiocyanate
Beaker 250 cm^3	1 No.	Aqueous ammonia
Bunsen burner	1No.	Copper(II) sulphate
Desiccator	1 No.	Dimethylglyoxime
Filtration apparatus	1 No.	Ethanol
Flask conical	1 No.	Hydrochloric acid (conc.)
Funnel	1 No.	Nickel(II) sulphate
Glass rod	1 No.	Nitric acid (conc.)
Pair of tongs	1 No.	Ammonium hydrogen sulphite solution
Rubber police-man	1 No.	Test solution
Sintered glass crucible (G-3)	1 No.	
Sintered glass crucible (G-4)	1 No.	
Tripod stand	1 No.	
Wash bottle	1 No.	

Watch glass	1 No.
Water bath	1 No.
Wire guaze	1 No.
Weight Box	1 No.

17.4 PROCEDURE

(A) Estimation of copper

I. Pipette out 50 cm^3 of test solution either assigned by your counsellor or prepared by you in a 500 cm^3 beaker. Add into it 5 cm^3 dilute HCI and 40-50 cm^3 ammonium hydrogen sulphite solution and ensure that smell of SO_2 prevails.

II. Boil the contents of the beaker gently on a water bath. Remove the flame and add 50 cm^3 NH_4SCN solution slowly. Keep stirring the precipitate with a glass rod intermittently. Let the solution, containing the precipitate, rest for 30-60 minutes.

III. Weigh an empty and cleaned sintered glass crucible (G-4) and note its mass. Filter the precipitate, obtained in step II, through this crucible first by draining off the supernatant liquid and then the precipitate with minimum quantity of liquid. This procedure of filtration keeps the precipitate away from clogging the pores of the crucible.

IV. Wash the precipitate with 2-3% NH_4SCN solution. Wait and check the filtrate. If any precipitate appears then filter the precipitate again through the crucible. Preserve the filtrate for estimation of nickel. Finally wash the precipitate several times with 20% ethanol until the precipitate is free from SCN^- ions.

V. Now heat the sintered glass crucible at a temperature of 100 – 120°C in an hot air oven for at least an hour.

VI. Cool the crucible in a desiccator and then weigh. Repeat the process of heating, cooling and weighing till the constant mass of the crucible with precipitate is obtained.

(B) Estimation of Nickel:

I. Evaporate the filtrate obtained in the estimation of copper in step IV on a water bath. Reduce the volume of the solution to nearly 100 cm^3 by evaporation. Now add 20 cm^3 concentrated nitric acid and 15 cm^3 concentrated HCl and heat to dryness on a water bath in a fume hood.

II. To the residue, obtained in step I, add 100 cm^3 of distilled water and dissolve the contents by shaking. Add 2-3 drops of methyl red indicator and then add 10% aqueous ammonia solution till smell of ammonia prevails and the colour of the methyl red changes to yellow.

III. Now add 40 – 50cm^3 of dimethylglyoxime solution with adequate stirring with a glass rod. Again add ammonia solution till the colour becomes yellow. Digest on a water bath atleast for 30 minutes.

IV. Filter the precipitate, obtained in step III, through a previously weighed sintered crucible (G-4). Drain out the supernatant liquid first and then the precipitate with minimum quantity of liquid.

V. Now wash the precipitate 2-3 times with 2-3% dimethylglyoxime solution. If any precipitate appears in the filtrate then filter it again through the crucible.

Wash the precipitate with 2-3% aqueous ammonia solution. Finally wash the precipitate with hot water.

VI. Heat the crucible and precipitate at temperature range 100 – 120°C in an air oven. Cool the crucible in a desiccator and then weigh it. Repeat heating, cooling and weighing till a constant mass of the crucible with precipitate is obtained.

17.5 OBSERVATIONS

i)	1st mass of empty crucible	= ..... g
ii)	2nd mass of empty crucible	= ..... g
iii)	1st mass of crucible + CuSCN	= ..... g
iv)	2nd mass of crucible + CuSCN	= ..... g
v)	1st mass of empty crucible	= ..... g
vi)	2nd mass of empty crucible	= ..... g
vii)	1st mass of crucible + $Ni(HDMG)_2$	= ..... g
viii)	2nd mass of crucible + $Ni(HDMG)_2$	= ..... g

17.6 CALCULATIONS

Calculations for Copper

Mass of CuSCN obtained = iv) – ii) = ...g = w g

From stoichiometry we know,

$CuSO_4.5H_2O \equiv Cu^{2+} \equiv CuSCN$

249.50 g $\equiv$ 63.54 g $\equiv$ 121.62 g

Hence, w g of CuSCN $\equiv \frac{249.5 \times w}{121.62}$ g of copper(II) sulphate.

This much copper(II) sulphate is present in 50 cm^3 of test solution. Hence, concentration of copper(II) sulphate in test solution

$$= \frac{249.50 \times w}{121.62} \times \frac{1000}{50} \text{ g dm}^{-3}$$

$$\text{Concentration of } CuSO_4.5H_2O = \frac{249.50 \times 1000 \times \text{mass of CuSCN}}{121.62 \times \text{volume of test solution}} \text{ g dm}^{-3}$$

Calculations for Nickel

Mass of $Ni(HDMG)_2$ obtained = viii) – vi) =..... g = w' g

From stoichiometry we find,

$NiSO_4 \equiv Ni^{2+} \equiv Ni(HDMG)_2$

154.69 g $\equiv$ 58.69 g $\equiv$ 288.91 g

Hence, w' g of $Ni(HDMG)_2 \equiv \frac{154.69 \times w'}{288.91}$ g of $NiSO_4$

This much nickel sulphate was present in 50 cm^3 of the test solution. Hence, concentration of nickel sulphate in the test solution,

$$= \frac{154.69 \times w'}{288.91} \times \frac{1000}{50} \text{ g dm}^{-3}$$

$$\text{Concentration of } NiSO_4 = \frac{154.69 \times 1000 \times \text{mass of } Ni(HDMG)_2}{288.91 \times \text{ volume of the test solution}} \text{ g dm}^{-3}$$

17.7 Result

You can report your result in the following manner:

Concentration of copper(II) sulphate in the test solution = ... g dm^{-3}

Concentration of nickel(II) sulphate in the test solution = ... g dm^{-3}

EXPERIMENT 18
PREPARATION OF ASPIRIN AND ANALYSIS OF A COMMERCIAL SAMPLE OF ASPIRIN.

Structure

18.1 INTRODUCTION

In the previous eight experiments you have learnt about the organic and inorganic quantitative analysis. In this and the forthcoming experiments you will be preparing certain molecules which have applications in our day to day life . The present experiment deals with preparation of a common drug , aspirin .

Aspirin is an antipyretic,an analgesic, and an anti inflammatory drug. It is probably the most extensively used analgesic drug. As an anti–inflammatory agent aspirin is used extensively in the treatment of arthritis. Now a day it is universally being recommended as a medicine which may prevent heart attack by checking blood clotting in the arteries. It is attributed to the fact that it effects platelets which are important for clotting of blood. On the negative side in excess doses it causes gastric problems like irritation of mucous membrane. It is also said to be responsible for brain disorder (Reye's syndrome) in people below age of 18 years.

In this experiment you will learn about preparation of aspirin from salicylic acid and also about the analysis of a commercial sample of aspirin .This experiment has two parts one for preparation and other for the analysis. However, the principle and procedure etc. for the two parts are given seperately. In the preparation part (section 18.2) you will learn how to perform " acetylation", an important organic reaction. As you will see later , acetylation reaction can be carried out in a number of ways. We are giving procedures for two methods. Depending upon the convenience of time and availability of chemicals, your counsellor can choose any of these. The analysis part (section 18.3) may be carried out with any of the available nonbuffered

commercial preparation of aspirin. In the next experiment you will learn about preparation of azo dyes.

Objectives

After studying and performing this experiment you should be able to :

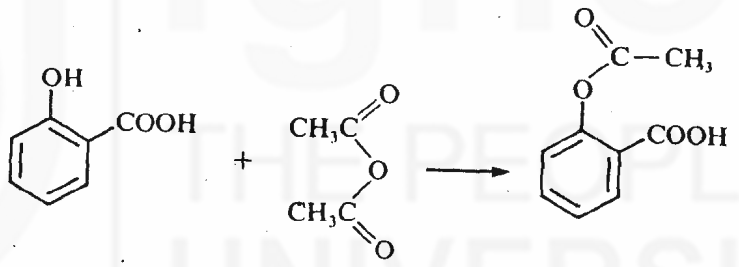
- prepare sparin from salicylic acid,
- explain the acetylation reaction and its mechanism, and
- analyse a commercial sample of aspirin and outline the uses of aspinin as a drug.

18.2 PREPARATION OF ASPIRIN

As said above, this experiment has to parts. In the first part, related to preparation of aspirin, you will learn about procedure and mechanism of acetylation of salicylic acid.

18.2.1 Principle

As the name, acetylsalicylic acid suggests, aspirin is acetyl derivative of salicylic acid. It is prepared by acetylation reaction of salicylic acid.



Salicylic acid Acetic anhydride Acetylsalicylic acid

Generally in an acetylation reaction the reactive hydrogen of hydroxy (alcohol or phenols) or amino (primary and secondary amines) functional group is replaced by $-COCH_3$ group.

$$\begin{matrix} R-OH \\ \text{or} \\ RNH_2 \end{matrix} \xrightarrow{\text{acetylation}} \begin{matrix} R-OCOCH_3 \\ \text{or} \\ RNHCOCH_3 \end{matrix}$$

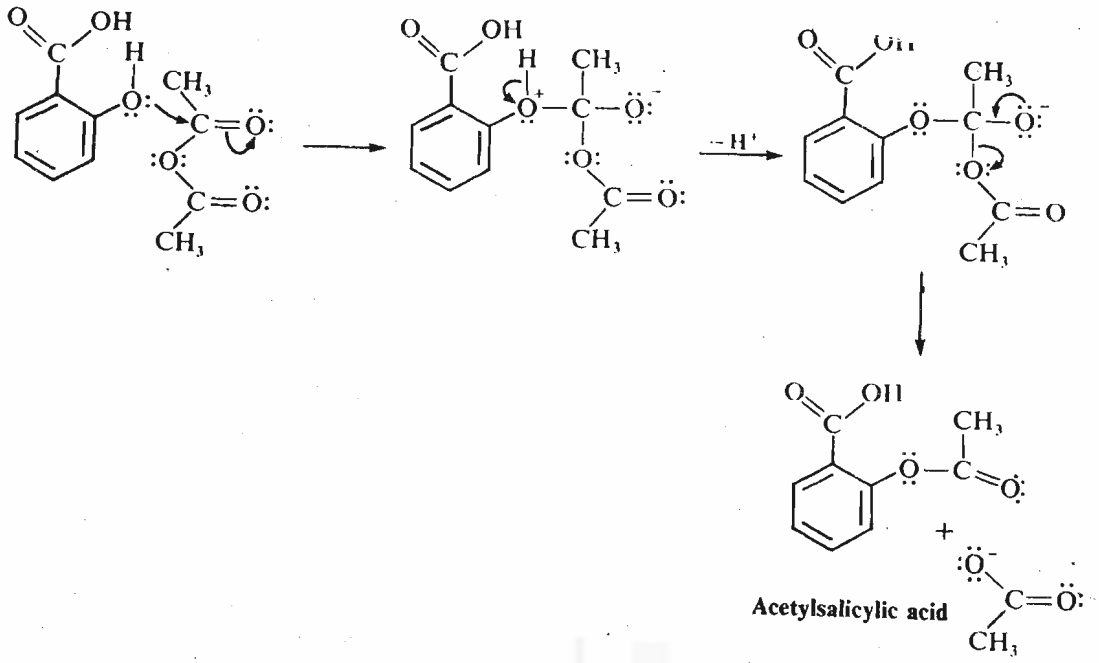
The acetylation of —OH group is equivalent to the esterification of acetic acid. It is so because the product obtained, R—$OCOCH_3$ is essentially an alkyl/aryl ester of acetic acid depending on whether R is alkyl or aryl group. Acetylation reaction can be accomplished in a number of ways. These are:

(i) with acetic anhydride in presence of a catalyst.

(ii) with acetyl chloride in presence of a base, like pyridine.

(iii) with a mixture of acetic acid and acetic anhydride.

Commercially, aspirin is prepared by method (i). We are giving procedure for both methods(i) and (ii), you may use any method as said above. The mechanism for acid catalyses acetylation of salicylic acid which may be represented as follows.



18.2.2 Requirements

Apparatus		Chemicals
Conical flask (100 cm^3)	2	Salicylic acid
Water bath	1	Acetic anhydride
Beakers (100 cm^3)	2	Sulphuric acid
		Acetyl chloride
Glass rod	1	Pyridine
		Alcohol

18.2.3 Procedure

Method i

1. Take 2.75 g (0.02 mole) of salicylic acid in a 100 cm^3 conical flask and to this add about 6 cm^3 of acetic anhydride (0.06 mol) and a few drops of conc. sulphuric acid.

   In this method acetic anhydride is taken in excess. It acts as acetylating agent as well as the solvent.

2. Swirl this flask in a water bath (temp = 50 - 60) for a few minutes till the solid material dissolves.

3. Leave the flask in water bath for about 10 minutes with occasional swirling.

4. Allow the solution to come to room temperature and then add about 50 cm^3 of ice cold water to it. You may even add crushed ice.

   Water is added to destroy the excess acetic anhydride, which gets converted to acetic acid.

5. Scratch the sides of flask with glass rod to induce crystallisation and filter the solid, so obtained.

6. Take about 10--15 mg (a speck) of the crude aspirin in a test tube and dissolve it in about 1cm^3 of alcohol. Add a drop of 1 % ferric chloride solution to it and observe the colour. Formation of intense colour indicates the presence of unreacted salicylic acid.

7. Recrystallise about half of the crude sample by ethanol\water solvent system. For this dissolve aspirin in minimum quantity of hot alcohol and to this add warm (50 - 60) water with constant swirling of the solution till a turbidity persists. If some crystals do form at this stage dissolve them by gently heating the solution.

8. Allow the solution to cool till the crystallisation is complete. Collect the crystals by vacuum filter and wash them with cold water and dry the crystal in the folds of filter paper and weigh them.

9. Determine the melting point of the recrystalised sample and report it.

Method ii

1. Dissolve 2.75g of salicylic acid to about 2 cm^3 of dry pyridine in a 100 cm^3 conical flask.

2. Quickly add about 2.5 ml of acetyl chloride to the above solution in small lots with constant swirling\shaking.

   Caution : This reaction is highly exothermic and the temperature of reacting mixture rises rapidly. Don't let the temperature go beyond about 60°C (unbearable to touch). You may cool the flask occasionally in cold water\under the tap.

3. Heat the mixture on a water bath for about 5 minutes.

4. Proceed exactly as in method 1 from step 4 onwards.

18.2.4 Results:

1. ..... g of acetylsalicylic acid was obtained from 2.75 g of salicylic acid.

   Theoretical yield = 3.6 g

   $$\% \text{ yield} = \frac{\text{yield obtained} \times 100}{\text{theoretical yield}}$$

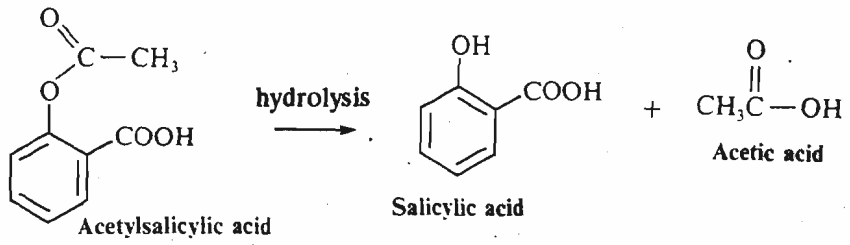
   = ...

2. The melting point of aspirin was found to be = ... °C.

18.3 ANALYSIS OF ASPIRIN

Purity of any compound is important for its action. It is all the more important if the compound happens to be a drug. The purity of aspirin prepared above can be checked qualitatively by the colour test given above. Development of colour with $FeCl_3$ indicates the presence of salicylic acid in the preparation but the question arises about the amount or percentage of salicylic acid in it. We need to undertake a quantitative examination for the purpose.

Similarly a commercial sample of aspirin may also have some amount of salicylic acid. This salicylic acid originates from the hydrolysis of acetylsalicylic acid. This hydrolysis brings in another impurity - acetic acid .You may have noticed a smell of vinegar on opening an old bottle of aspirin tablets. This is due to acetic acid formed during hydrolysis.



The presence of salicylic acid, whether as an impurity in the preparation or consequence of hydrolysis, is not desirable. Incidentally salicylic acid also is an analgesic but is not as safe as aspirin, because the free —OH group causes severe mucousal irritation and gastric problems. Excessive use may even cause gastric ulcers. Further, you know that the effectiveness of a drug depends on its proper dosage besides the purity. The amount of aspirin per tablet is normally marked on the packing. You would like to know that whether the tablet you are consuming for curing your headache has enough of aspirin in it. Let us learn how do we make such determination for any laboratory/ commercial preparation of aspirin.

18.3.1 Principle :

The amount of aspirin in any preparation can be determined by a number of methods. These include simple titrimetry, conductormetry, potentiometry and colorimetry etc. . We are providing the detailed principle and procedure for the titrimetric method.

To determine aspirin titrimetrically it is first hydrolysed with an excess but known amount of strong alkali solution, which generates equivalent amounts of salicylic and acetic acid(eq.) These neutrlise part of the alkali and the remaining alkali is back titrated with a standard solution of an acid.

Normally in a titration we add the standard solution of the titrant (from the burette) to the titrand (in the conical flask) till the two react stoichiometrically ; marked by a colour change of the indicator. In certain situations excess of the titrant is added to the titrand. After the reaction is over the excess of the titrant is titrated with another reagent. This arrangement of performing the titration is called as residual or back titration.

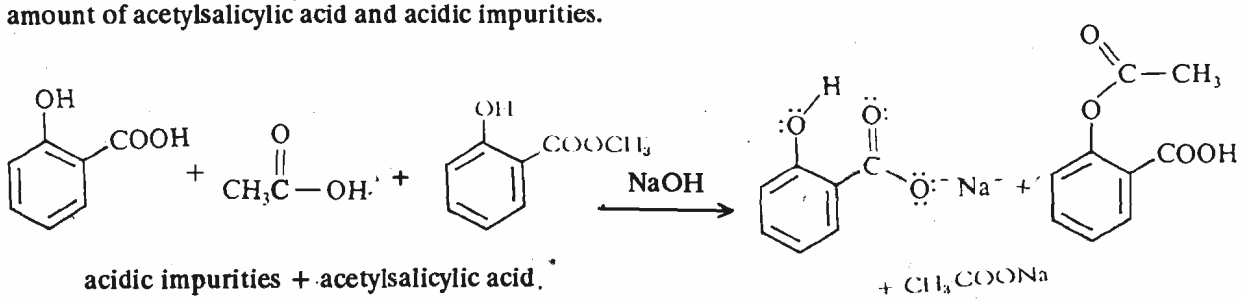
As we can see from the above equation, each mole of acetylsalicylic acid would neutralise 2 moles of NaOH. We can represent the overall chemical equation as;

$$COOHC_6H_4OCOCH_3 + 2NaOH \longrightarrow NaOOCC_6H_4OH + CH_3COONa$$

Knowing the amount of NaOH consumed by acetylsalicylic acid we can estimate the amount of acetylsalicylic acid. It would be half the amount (in moles) of NaOH consumed. You may raise a question here that the impurity of salicylic acid and/or acetic acid (if present) would also react with NaOH and consume some of it. Your question is quite valid and in fact the mols of NaOH consumed is equal to the moles of acidic impurities plus twice the moles of acetylsalicylic acid . If a given sample contains 'a' moles of acetylsalicylic acid and 'b' moles of acidic impurities, then ;

the moles of NaOH consumed in back titration = 2a + b moles

But, we need to know 'a', the moles of acetylsalicylic acid. How do we get over this problem ? To come out of this problem we have to perform yet another titration of aspirin with alkali in alcoholic medium. Under alcoholic conditions the alkali does not hydrolye the acetyl group significantly (the reaction is too slow).We can directly titrate the -COOH groups. Such a direct titration would provide an estimate of total amount of acetylsalicylic acid and acidic impurities.



You may note here that in this titration each mole of acetyl salicylic acid would

consume only one mole of NaOH. That is ,

the moles of alkali consumed in direct titration = a + b moles

From the results of the two titration we can eleminate"b" and get the value of 'a', the amount of acetylsalicylic acid.

amount of acetylsalicylic acid	=	amount of NaOH used in titration 1 (back titration)	–	amount of NaOH used in titration 2 (direct titration)
		= (2a + b) – (a + b)		= a moles

Commonly, starch is employed as binder. The use of commercial proparation containg buffers is not recomended for this experiment.

This may be sounding quite tedious an excercise. You may relax because unexposed commercial preparation normally do not have appreciable amount of acetic / salicylic acid. But they do have impurities of binders and buffers. Actually the manufacturers use some kind of binder to keep the tablet intact. These binders are usually inert and do not interfere in the titration. However, the impurity of buffer is not desirable in this experiment. This means that 'b' in the above formula is negligible for commercial tablets and we can quite a good estimate of the amount of acetyl salicylic acid found by method of back titration. This will provide you the amount of aspirin in the given commercial tablet. However, if you are using your own preparation then you will have to perform both, the direct as well as back titration.

The British pharmacopia, also recomands aspirin assay by back titration.

In fact you are going to perform experiment involving hydrolysis and back titration and in this

$$\text{amount of acetylsalicylicacid} = \frac{\text{amount of NaOH used in back titration}}{2}$$

18.3.2 Requirements:

Apparatus		Chemicals
Apparatus Burette (50 cm^3)	1	NaOH
Pipette (25 cm^3)	1	H_2SO_4
Conical flask (100 cm^3)	3	Phenol red (indicator)
Measuring flask 250 cm^3	2	Aspirin sample
Conical flask 250 cm^3	1	

Solution Provided.

1. 1*M* **NaOH solution :** It can be prepared by dissolving 4 g NaOH in water in 100 cm_3 volumetric flask

2. **0.05** *M* H_2SO_4 **solution.** This is prepared by taking 2 cm^3 sulphuric acid in water in 1 dm^3 volumetric flask.

18.3.3 Procedure :

1. Weigh accurately 3-4 tablets of aspirin (about 1.5 g) and transfer them to a 250 cm^3 conical flask.

2. Add 25.0 cm^3 of 1*M* NaOH solution and 25cm^3 of distilled water to it and gently heat the mixture for about 10-15 minutes.

This is done so as to hydrolyse the aspirin. Do not boil the solution and avoid spilling.

3. Allow the reaction mixture to cool and transfer it to a 250 cm^3 standard flask.Rinse the conical flask twice with distilled water and transfer the washings also to the standard flask. Ensure that whole of the reaction mixture is transferred to the standard flask.

4. Make up the volume to the calibration mark by adding more of distilled water and thoroughly mix the solution.

5. Transfer 25 cm^3 of this solution with help of a pipette to a 100 cm^3 conical flask and add a 2-3 drops of phenol red indicator.

6. Titrate this against 0.05 *M* sulphuric acid solution (taken in burette). The titration is marked by the change of colour from pink to orange. Record your observations in Observation Table I.

7. Repeat step no. 6 till you get at least two concordant readings.

8. Take 25 cm^3 of 1*M* sodium hydroxide solution with the help of a pipette and transfer it to another 250 cm^3 measuring flask. Dilute the solution to calibration mark with distilled water.

9. Transfer 25 cm^3 of this solution with a pipette to a 100 cm^3 conical flask. Add 3 drops of phenol red indicator and titrate against 0.05*M* H_2SO_4 solution (taken in burette). Record your observations in Observation Table II.

10. Repeat step 9 till you get two concordant readings.

18.3.4 Observations

mass of weighting tube $= m_1$ =.....g

mass of weighting tube + 3 tablets of aspirin $= m_2$ =.....g

mass of aspirin taken $= m_2 - m_1 = m$ g

Observation Table I
Titration of reaction mixture Vs 0.05*M* H_2SO_4 solution

Sl. No.	Volume of reaction mixture in cm^3	Burette reading initial	final	Volume of 0.05*M* H_2SO_4 used in cm^3 (final–initial)

Concordent reading =.....

Observation Table II
Titration of NaOH solution Vs 0.05 *M* H_2SO_4 solution

Sl. No.	Volume of NaOH solution in cm^3	Burette reading initial	final	volume of 0.05*M* H_2SO_4 used in cm^3 (final–initial)

Concordent reading = ...

18.3.5 Calculations:

Reaction involved in the titration

$$2NaOH + H_2SO_4 \longrightarrow Na_2SO_4 + 2H_2O$$

Let, molarity of acid, H_2SO_4 $= M_A$ (Provided)

molarity the of base, NaOH $= M_B$

volume of the base, NaOH $= V_B$

volume of the acid, H_2SO_4 $= V_A$

Molarity equation $= \frac{M_A \times V_A}{M_B \times V_B} = \frac{1}{2}$

$= 2M_AV_A = M_BV_B$

For titration of NaOH (standard) Vs sulphuric acid

Molarity of acid, M_A =..... (Provided)

Volume of acid used, V_A = ... (from Observation Table II)

Volume of NaOH taken, V_B = 25.0 cm^3

Molarity of NaOH, $M_B = 2M_AV_A/25$ = ... mole

For titration of reaction mixture Vs sulphuric acid

Molarity of sulphuric acid solution, M_A =..... (Provided)

Volume of sulphuric acid used, V_A =..... (from Observation Table I)

Volume of NaOH taken, V_B = 25.0 cm^3

Molarity of NaOH, $M_B' = 2M_AV_A/25$ =..... mole

⇒ Amount of NaOH (in mole) consumed for neutralizing the hydrolysis products of aspirin

⇒ $(M_B - M'_B)$ moles

⇒ Amount of aspirin (in mole) $= \frac{M_B - M'_B}{2}$ moles

Amount of aspirin (in g) $= \frac{M_B - M'_B}{2} \times 180.16\ g = Z\ g$

M_m of aspirin = 180.16 g mol^{-1}

mass of aspirin (from titration) $= Z$ g

mass of aspirin (weighed from tablet) $= m$ g

percentage of aspirin in the given sample $= (Z/m) \times 100$

17.3.6 Result

The given sample contains ..... percent of aspirin.

EXPERIMENT 19
PREPARATION AND USE OF METHYL ORANGE–AN AZO DYE

Structure

19.1 INTRODUCTION

In the previous experiment you have learnt about synthesis and analysis of a common analgesic drug, aspirin. In this process you have learnt about acetylation reaction and you have yourself performed one such reaction. In this experiment we extend our endeavour of synthesizing organic molecules to another very important class of compounds viz. dyes.

You would be preparing methyl orange as a sample of azo dyes, and would be using it to dye a piece of cloth. In this process you would learn about another important organic reaction i.e. diazotisation. Since methyl orange happens to be an acid base indicator you will be checking this property for the prepared sample. In the next experiment you will be learning about yet another important class of organic compounds with wide range of applications–polymers.

A brief account of dyes in general and azo dyes in particular has been included in the next section before the principle and procedure for the actual experiment.

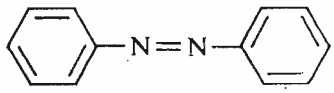
Objectives

After performing this experiment you should be able to:

- prepare an azo dye,
- use the prepared dye to dye a piece of cloth,
- explain the diazotisation reaction of primary aromatic amines, and
- explain 'coupling reaction' in context of azo dyes,

19.2 AZO DYES

Dyes may be defined as the chemical substances which when applied to a fibre or a surface impart a relatively permanent colour to it. These are distinct from coloured substances which by themselves may be coloured but may not be able to impart a colour to other things.For example, azobenzene (I) is a coloured compound but is not a dye whereas p-aminoazobenzene (II) is coloured and also acts as a dye.



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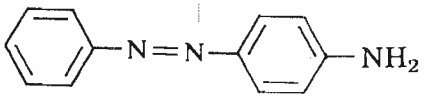


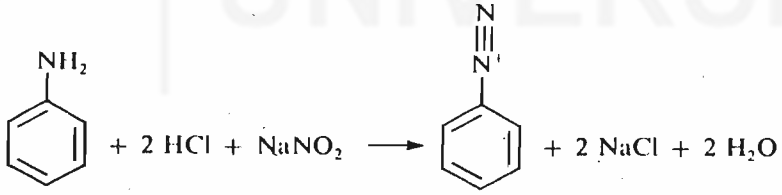
II

Dyes are classified into a number of groups on the basis of their chemical structure or their mode of application. Azo dyes are probably the largest category of dyes. The number of azo dyes available now a days out numbers all the other dyes put together. This class of dyes owes its name to the presence of one or more azo (-N = N) functional group in its members. These are prepared by diazotisation of primary aromatic amines followed by coupling of a suitable coupling agent. The coupling agents are normally phenols or amines (substituted or unsubstituted). The variations possible in the diazotised component and/or the coupling component account for the enormously large number of azo dyes. The details of diazotisation and coupling reaction are given under the principle of the experiment.

Azo dyes can be applied to a fiber by a number of different modes. This makes several sub-groups in azo dyes like, direct azo dyes (applied directly), disperse azo dyes (the insoluble dye is dispersed by a carrier substance) or ingrain azo dyes (dyes developed in the fibre itself) etc., etc.The dye which you are going to prepare i.e. methyl orange belongs to direct azo dyes group. Let us learn in this experiment what is diazotisation and coupling reaction and how to we prepare and use an azo dye.

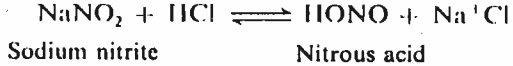
19.3 PRINCIPLE

As stated above, the preparation of an azo dye involves two stages known as diazotisation and coupling. In the diazotisation step a primary aromatic amine reacts with nitrous acid (HONO) to form a diazonium salt.The nitrous acid is generated **in situ** by sodium nitrite and a mineral acid like HCl.

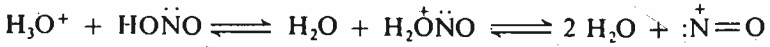


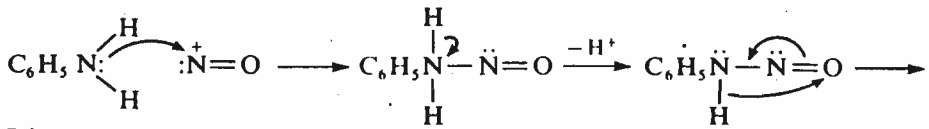
Diazonium salts are very important intermediates and provide convential routes to bring about a number of organic transformations. Refer to any standard text book on organic chemistry for more details on their versatility.

The mechanism of diagati-sation reaction is as follows:

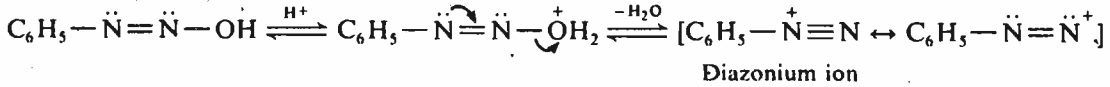


Sodium nitrite Nitrous acid





Primary aromatic amine



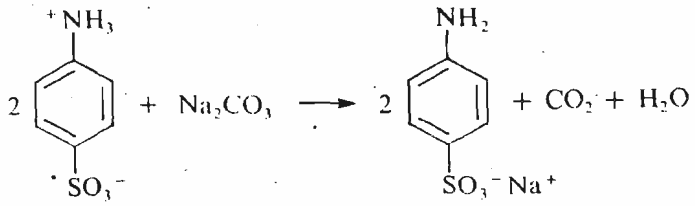
Diazonium ion

These diazonium salts are quite unstable so the reaction is carried out at low temperatures (below 5°C). Further, owing to their instability these are normally not isolated from the solution and the second (coupling) component is added directly to the diazonium salt solution.

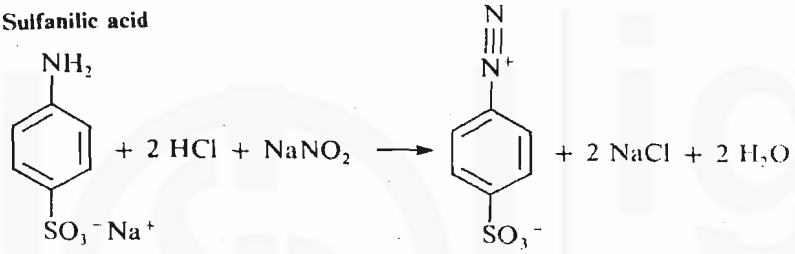
In the coupling stage the diazonium salt prepared in the first step is made to react with acidic (if amine) or basic (if phenol) solution of the coupling agent.

Benzene diazonium chloride | N,N-Dimethylaniline | A diazo compound

In the preparation of methyl orange sulphanilic acid is diazotised and coupled to N,N– dimethylaniline . The reactions may be represented as follows :



Sulfanilic acid

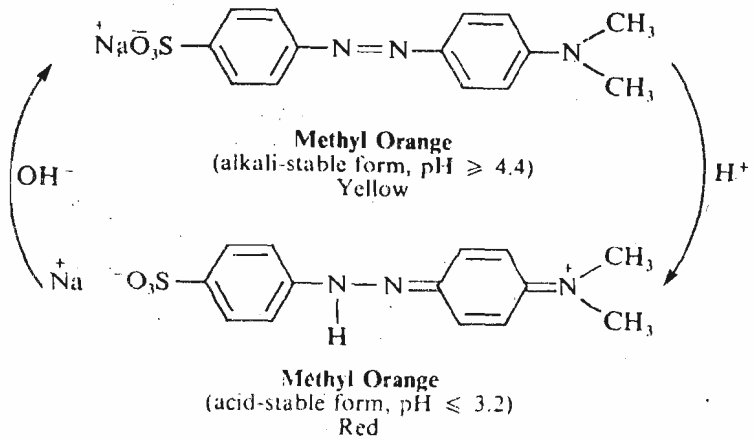


Diazotized sulfanilic acid

Diazotized sulfanilic acid | N,N-Dimethylaniline | Methyl orange

As regards to application of dye to fibre, dying with direct dyes (which we are preparing) is quite easy. In this process the acidic or basic form of the dye is dissolved in water, the solution is heated and the cloth to be dyed is immersed in hot solution, the polar groups on the dye help the dye to attach itself to the fibre by interacting with the polar groups of the fibre. Silk and wool have a number of polar groups (in their polypeptidic chains). Hence these bind very strongly to such dyes.

In addition to acting as a dye, methyl orange acts as a good acid base indicator. This properly is a consequence of distinctly different colour of methyl orange in weakly and strongly acidic condition. The chemical structures of methyl orange in the two forms are as given below:



Methyl Orange
(alkali-stable form, pH ≥ 4.4)
Yellow

Methyl Orange
(acid-stable form, pH ≤ 3.2)
Red

The indicator range of methyl orange is 3.3 – 4.4 which means that at pH values below 3.3 the acid form is predominant while at pH above 4.4 the basic form is predominant and within the

19.4 REQUIREMENTS

Apparatus		Chemicals
Ice bath		N,N-Dimethyl aniline
Beakers 400 cm^3	2	Sulphuric acid
Buchner funnel	1	Sodium nitrite
Conical flask 250 cm^3	2	Hydrochloric acid
Test tube		Acetic acid
		Sodium hydroxide
		Sodium chloride
		Sodium carbonate

Solutions Provided

1. **10% solution sodium hydroxide :** This solution is prepared by dissolving log NaOH in 100 cm^3 water.
2. **Saturated solution of sodium chloride :** It is prepared by dissolving ecess amount of NaCl in water.
3. **2.5% solution Sodium carbonate :** It is prepared by dissolving 2.5 g $Na_2 CO_3$ in 100 cm^3 of water.
4. **10% Sodium of sodium carbonate:** This solution is prepared by dissolving 10 g Na_2CO_3 in 100 cm_3 of water.

19.5 PROCEDURE

The procedural instructions have been given below in sequential order. You are expected to go through the instructions and prepare a broad mental outline of the same.

19.5.1 Preparation of Dye

Diazotisation step

1. Take 4.8 g (mole) of sulphanilic acid monohydrate in about 50 cm^3 of 2.5 % sodium carbonate solution in a 250 cm^3 conical flask and boil the solution till sulphanilic acid dissolves.
2. Cool the flask under the tap and add 1.9 g of sodium nitrite to it and stir to dissolve it.
3. Cool the flask in ice bath (you may even add clean crushed ice to the flask itself) and carefully add about 5 cm^3 of concentrated hydrochloric acid with constant stirring/swirling.
4. A solid material (suspension) starts separating slowly. This is 4-diazobenzene sulphonic acid. It is quite stable at low temperature.

Coupling step

5. Take 3.2 cm^3 of dimethylaniline and 2.5 cm^3 of glacial acetic acid in a test tube and thoroughly mix the two. You will get a solution of dimethylaniline acetate.
6. Add this solution slowly with constant stirring to the suspension of diazonium salt prepared above.
7. Continue stirring, you will observe the separation of a red coloured dye.

8. Add about 35 cm^3 of 10 % sodium hydroxide solution stir and boil the solution till most of the dye dissolves.

9. Allow the solution to cool slowly. Filter it in the buchner funnel and wash the product with saturated solution of sodium chloride. 10. Dry it in the folds of filter paper. Weigh it, and report the yield

19.5.3 Dyeing of Cloth

1. Take about 100 mg of methyl orange dye in 30 cm^3 of water containing 1.0 cm^3 of 10 % sodium sulphate solution and 10 drops of 10 % sulphuric acid in a 100 cm^3 beaker.

2. Dip a small piece of silk cloth or a small length of wool in the above dye bath and heat the bath to almost boiling.

3. Remove the cloth/wool from the bath after it has stayed for about 5 minutes at the boiling temperature.

4. Allow it to cool, wash thoroughly under running tap water and dry it.

19.5.3 Indicator Properties of Methyl Orange

1. Take roughly 10 cm^3 of distilled water in a 100 cm^3 beaker or conical flask and to this add 1 drop of dilute hydrochloric acid and a small crystal of methyl orange and shake the solution. It acquires red colour due to the acid stable form indicated above.

2. To this add 3 - 4 drops of 10 % sodium hydroxide solution (or a pallet NaOH) mix thoroughly. The colour changes to yellow (the alkali stable form).

19.5 Results

i) ..... g of methyl orange was obtained.

ii) It dyes silk and wool (sample enclosed).

iii) It's acid - base indicator property has been verified.

EXPERIMENT 20
PREPARATION OF NYLON 66 - A CONDENSATION POLYMER

Structure

20.1 INTRODUCTION

In the previous experiment you have learnt about preparation and use of azo dyes. In this process you have learnt about diazotisation reaction.In the present experiment you are going to prepare a sample of nylon 66 as an example of yet another important class of organic compounds viz. polymers.

In past few decades the developments in the field of polymers have dramatically changed our way of living. The way we dress ourselves, the way we pack our things, the way we decorate/furnish our household, everything has changed. Owing to their wide variety and versatility they have entered into practically every sphere of our day to day life. Polythene, used in carry bags; polyesters, the synthetic fabrics ; poly vinyl chloride (PVC) used in water proof coverings ; melamines, the unbreakable crockery ; plastics, used for a whole range of materials have become part of our day to day vocabulary. The large number of available monomers and the ways in which they can combine together to form polymers provides for such a wide range of polymeric molecules,

In the preparation of nylon 66 you will learn about how small molecules " condense " together to make larger molecules i.e. condensation polymerisation. In the next section we have included a brief introduction to polymers, in general and nylon, in particular. In the next experiment, you will learn about another important class of organic compounds namely cosmetics and you will be preparing a sample of a face cream.

Objectives :

After performing these experiments you should be able to :

- prepare a sample of nylon 66
- explain condensation polymerisation, and
- list various applications of the polymer prepared.

20.2 POLYMERS : AN INTRODUCTION

The term 'POLYMERS' has been derived by the synthesis of two Greek words, "poly" meaning "many" and "meros" meaning "part".i.e. a molecule made up of many

parts. The number of small molecules that makes the polymer are called Monomers (monos meaning single and meros meaning part). The polymers can be of natural origin or synthetic.Natural polymers like proteins, nucleic acids and cellulose are the basic building blocks of living organisms.

The synthetic polymers, used in day to day life, may be classified on the basis of their properties as elastomers, fibres or plastics. Elastomers get elongated under stress and reform their shape on removing the stress. Fibres are thread like polymers which can be woven into fabrics e.g. nylon, dacron etc. These are light and have high tensile strength. Plastics, on the other hand are intermediate between elastomers and fabrics. They can have variety of properties from highly flexible thin sheets to tough solid material of polypropyline.

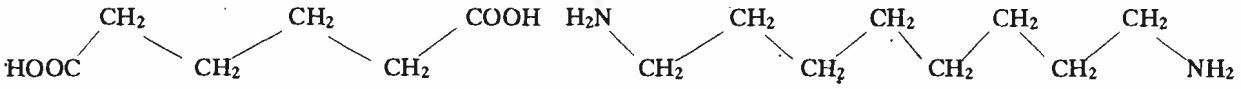
Another classification of polymers is based on the way the monomers are joined together to make a polymer. In addition, polymers the monomers are normally unsaturated and simply add on by 'opening up' their double bonds where as in condensation polymers the two monomers join together and eliminate a small molecule (like H_2O).

Nylon was the first completely synthetic fibre developed by a Carruthers of Du Pont laboratories in 2030. It is basically a polyamide formed by the condensation of monomers containing -- NH_2 and --COOH groups, respectively.

Now a days there are a whole range of nylon polymers. These are named according to the numbers of carbon atoms in the monomers making the polymer. For example the polymer you are going to prepare, nylon 66, has 6 C atoms in each of the monomers. While nylon 6 10 has 6 C atoms in the diamine and 10 carbon atoms in the diacid. In the next section you will learn about the reactions involved in the preparation of nylon 66 .

20.3 PRINCIPLE

As said above nylon 66 is a polyamide obtained by condensation polymerisation. The monomers of nylon 66, are adipic acid (I) and hexamethylene diamine (II). Adipic acid is a dicarboxylic acid with 6 carbon atoms while hexamethylenediamine is a diamine with same number of carbon atoms.



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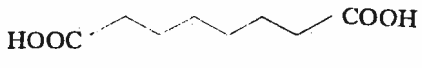
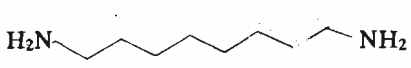


(I)

Or



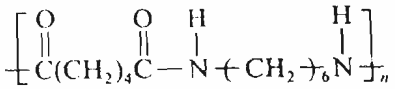
(II)

Adipic acid contributes toward the sharp taste of beets.

Let us see how do we get the polymers from the monomers. The polymerization reaction can be visualised as follows. First of all one molecule each of the monomers react to produce a dimer (dimer is a molecule made from 2 monomers). A carboxylic acid group condenses with an amine by eliminating water molecule to produce an amide.

The resulting dimer has got two functional groups one carboxylic acid and the other amino group. This dimer can further combine with diamine and diacid on the acidic and amino functional group respectively or with another dimer molecule. This process continues to generate the polymer.

The proteins in our body also contain such linkages formed by condensation of amino and carboxylic acid groups of different amino acids.



(Nylong 66)

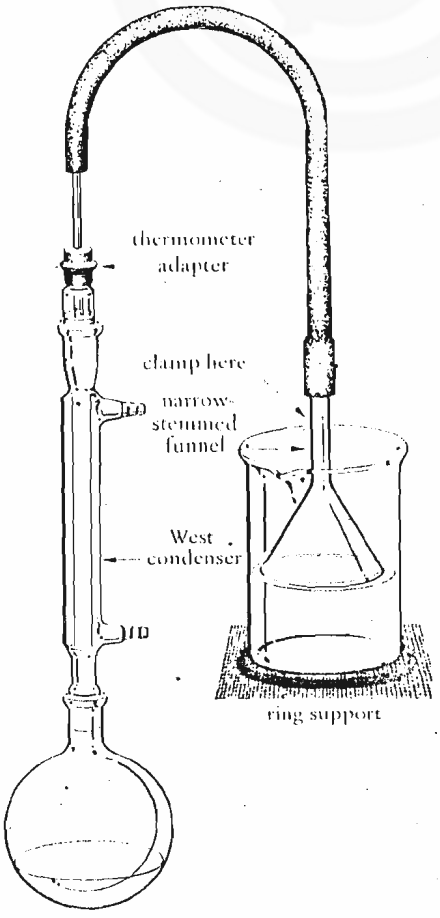
The condensation of monomers to form the polymer can be done in a number of ways. You would be using the method of interfacial condensation. In this technique the polymerization is made to take place at the interface of an organic and an aqueous medium. This technique is good for the reactants with quite reactive functional groups, which is true in our case.

The diamine is dissolved in water and the diacid chloride is dissolved in an organic solvent like chloroform or carbon tetrachloride. The aqueous layer is carefully poured on the top of organic layer. Diamine being soluble in both the solvents diffuses from aqueous to organic layer and react with the reactive diacid chloride at interface and produces the insoluble polymer. This polymeric layer checks further diffusion of the diamine and the reaction stops. The by-product, HCl,formed during condensation diffuses back into the aqueous phase and gets neutralized by the base. The polymer can be removed with the help of a forcep or a glass rod and the process continues. The polymer is removed continuously till the reactant are exhausted.

At industrial scale production the polymer is not pulled out, as stated above , but the two solution are thoroughly agitated so as to form an emulsion. This leads to large increase in interfacial surface area. As a consequence the rate of polymerization increases and a good amount of polymer is obtained in short time.

20.4 REQUIREMENTS :

apparatus		chemicals
Round bottom flask 50 cm^3	1	Adipic acid
Air condenser	1	Hexamethylene diamine
Beaker 100 cm^3	2	Thionyl chloride
Beaker 400 cm^3	1	Dimethyl formamide
		Carbon tetrachloride
		Sodium hydroxide
		Alcohol



20.5 PROCEDURE :

Preparation of adipoyl chloride :

1. Set up the apparatus as shown in the figure. For this take a 50 cm^3 round bottom flask and fit an air condenser on to it. Take a glass fannel and inset its sleen into a piece of rubber tubing. In sent a glass tube in the other end of the rubber tube and fit it on the air condenser as shown in the Fig. Given in martgin. Put the inverted funnel into a beaker containg water or NaoH solution.

2. Take 1g of adipic acid in 3-4 drops of dimethyl formamide in the flask and add 1 cm^3 of thionyl chloride dropwise with constant stirring.

3. Heat the flask on water bath for about 15 minutes. By this the evolution of gas ceased and the solid will disappear.

4. Allow the flask to cool a little and then add about 30cm^3 of CCl_4 to it. Mix thoroughly to dissolve the product obtained.

5. Transfer this solution to a 100 cm^3 beaker. Wash the flask throughly with additional 20 cm^3 of CCl_4 and transfer the washings to the beaker.

6. In a separate 100 cm^3 beaker take 1.1 g of hexamethylene diamine and 0.75 g (6-7 pallets) of NaOH in 25 cm^3 of water and mix to dissolve them.

7. Carefully transfer the aqueous solution of hexa methylene diamine to the beaker containing adipoyl chloride.

8. You will observe the formation of a film of nylon 6, 6, at the interface of the two liquids. Carefully insert a glass rod or a copper wire into the solution and pull out the polymer formed. You may even use a forceup to do so.

9. Wrap the polymer around a clean test tube as shown in the diagram. Rotate the test tube to pull more and more of nylon.

CAUTION ! Avoid handling the polymer with hands at this stage because the reactants specially adipoyl chloride and the solvent (CCl_4) both are harmful.

Do not pour the unreacted polymerization mixture in the sink. Stir it with a glass rod till there is no more polymerization. You may recover this crop of the polymer also and discard the rest of the solvent as usual.

10. Wash the polymer thoroughly with water. For this place the test tube, with polymer wrapped around, under the running tap water for about 5 minutes. Alternatively you may first wash the polymer with 50 % aqueous alcohol (alcohol:H_2O ::1:1) followed by tap water.

11. Dry the polymer in air or in the folds of filter paper. Weigh it and report the yield.

12. Submit a sample of the polymer so prepared to your counsellor.

Put 1-2 crystals of azobenzene, if available, to this solution and mix to dissolve. This will enhance the visibility of the interface of two liquids.

OR

You may add a drop of phenolphthalein to the aqueous layer containing hexamethylenediamine.

You should add the aqueous solution slowly along the walls of the beaker. Taking care, not to allow mixing of the solvents.

20.6 RESULT

..... g of nylon 66 was obtained from ..... g of adipic acid.

EXPERIMENT 21
PREPARATION OF FACE CREAM

Structure

21.1 INTRODUCTION

This experiment has been written to understand about different cosmetics with special reference to the preparative methods of face cream. This has been written in a very simple way so that anyone without any technical education or experience can start making various types of beauty creams without any complicated or expensive machinery.

Objectives:

After studying and performing this experiments you should be able to:

- describe the cosmetics and brief history of face cream,
- explain the mechanism of action of creams and lotion on the skin, and.
- prepare various forms of creams.

21.2 COSMETICS

Cosmetics are considered to be the preparation used for beautification of skin, hair or fingernails. The important critarion to market these preparations is that it should be safe and free from any dangerous side effects. In general, cosmetics are composed of oils, greases, fat waxes, emulsified agents, water, chemicals and perfumes. To combine some of these ingredients with some of the other requires certain definite and well studied procedures.

It is hard to confine the field of cosmetic because truely speaking it is a very wide field. It includes aerosols, antibiotics, cosmetic colours, emulsions, perfume formation, pharmacology and toxicology, preservation, in addition to popular household cosmetic products. The scope of cosmetics is specifically discussed by cosmetologists, Industrialists and the Government. Thus mirrors, brushes, razors or breast binders are outside the field of cosmetics, though they too help to beautify the human body. The U.S.A. Govt. restricts the toilet soap to be considered a cosmetic product and some cosmetologists have the same view regarding deodorants. Since deodorants have some type of fragrance and toilet soap are approximately as effective as cleansing creams, we may include both under cosmetics. In general, only those preparations are called cosmetics which have a pleasing and beautifying effect to human body.

21.3 CLASSIFICATION OF COSMETICS

The classification of cosmetic product varies in many ways. Generally all cosmetics fall into the following categories.

1. Fragrance products
2. Grooming preparations
3. Make up preparations
4. Treatment preparations
5. Hair preparations
6. Manicure preparations

Another way of classifying them is the basis of body part for which these are used such as -

1. Hair
2. Eye
3. Lips
4. Mouth
5. Skin
6. Nail
7. Dental
8. Bath
9. Face
10. Miscellaneous

Whatever be the basis of their classification, they are as follows -

1. Creams and Lotions
2. Powders and Rouges
3. Shampoos and bath preparations
4. Hair preparations
5. Shaving preparations
6. Mouthwashes etc.
7. Lipsticks
8. Nail preparations
9. Dental preparations
10. Eye preparations
11. Antiperspirants and deodorants
12. . Baby preparations

In this experiment, we are more concerned about the preparation of face creams, so we will deal about their descriptions, ingredients and preparative methods.

21.4 CREAMS

The preparation and application of creams dates back to the earliest time when these were prepared by digesting aromatic gum resins, roots, flowers etc. with fats and oils. The use of salves and ointments had also been very common for preservation and beautification. Later developments erupted with the result that water too became one of their constituents and the different types of creams that we see today like cold creams, milk of roses and cucumber creams, vanishing creams etc. are nothing but

the emulsification of fats, oils, bees wax and water. All other creams except the last one have emollient properties. Vanishing cream itself has no importance or has the least importance when used alone. However, undoubtedly it plays an important role when used in conjunction with beauty aids like face powder etc.

Cold creams are the emulsification products of fats. At one time almond oil, linolin and white wax, formed the basis of these preparations but these have now been replaced by liquid paraffin to avoid rancification on keeping.

21.4.1 Mechanism of action

The action of cold creams can well be understood like this: the coldness is caused due to the evaporation of water, present as a constituent in it. As we know evaporation causes cooling, this is how we feel coldness due to the application of this cream. It is, therefore, used generally in summer. Indeed, it is doubtful if such products have any intrinsic worth other than that of a protective agent against wind and sun.

21.4.2 Preparation of creams

Following ingredients and procedures will be used for the preparation of different types of creams.

a) **All purpose cream**:- The ingredients and their proportion will be as follows -

		Parts
1.	Lauryl alcohol	110
2.	Bees Wax	80
3.	Paraffin	70
4.	Mineral oil	10
5.	Sodium lauryl sulphate	10
6.	Triethanolamine	4
7.	Water	456
8.	Preservative	10
9.	Perfurme	

In one beaker heat first four ingredients to 82°C (180°F). in second beaker add sodium lauryl sulfate, preservative and triethanolamine to water and heat to the same temperature. Add mixture of Beaker 1 slowly to the 2nd beaker with continuous stirring. Continue stirring until the mixture has cooled to 60°C then add perfume. A homogenous paste of cream is obtained which can be transferred in a bottle.

b) **Night Cream**:- The ingredients and their preparation for this cream will be as follows:-

		Parts
1.	Mineral oil	280
2.	Olive oil	45
3.	Lanolin	125
4.	Stearic acid	40
5.	Spermaceti	65
6.	Cetyl alcohol	125
7.	Triethanolamine	109
8.	Water	400
9.	Preservative	10
10.	Perfume	

Heat water to 70°C with triethanolamine in one beaker. Heat first six ingredients together to some temperature in the second beaker. Mix the contents of beaker 1 to the beaker 2 with continuous stirring until mixture cools to 50°C than add preservative with stirring and finally add the perfume. Store the paste in a bottle and use it as a night cream.

C) **Cold cream**:- The contents of cold cream used in its preparation are as follows:-

		Parts
1.	Stearic acid	30
2.	Anhydr lanolin	20
3.	White bees wax	16
4.	Terpineol	1/5
5.	White mineral oil	33
6.	Triethanolamine	4
7.	Propylene glycol	16
8.	Water	95
9.	Perfume	

Melt the stearic acid, lanolin and bees wax in the mineral oil, heat to 70°C and then add the terpineol. Heat the water to 70°C in a separate beaker, add the triethanolamine and then add this solution to the hot mixture of wax and oil. Stir vigorously until a creamy emulsion is obtained. Add the perfume to the propylene glycol and add this solution to the emulsion. Continue stirring until the emulsion is smooth and quite viscous and then stir occasionally until room temperature is reached.

d) **Vanishing cream**:- A vanishing cream is essentially a stearic acid soap with excess suspended stearic acid dispersed in water.

		Parts
1.	Stearic acid	140
2.	Lauryl alcohol	30
3.	Triethanolamine	7
4.	Sodium lauryl sulfate	5
5.	Glycerine	50
6.	Water	770
7.	Preservative	10
8.	perfume	

Mix the first 3 ingredients and heat to 82°C. Add the sodium lauryl sulfate and the glycerin to the water and heat to same temperature and add to previous mixture with stirring. Continue stirring until cools the mixture to 60°C than add preservative and perfume.