MSCCH-505

**M. Sc. I Semester**

CHEMISTRY LAB-I: INORGANIC AND ORGANIC

SCHOOL OF SCIENCES

DEPARTMENT OF CHEMISTRY

UTTARAKHAND OPEN UNIVERSITY

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UNIT 1: QUALITATIVE ANALYSIS

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1.1 OBJECTIVES

In this unit learner will be able to

- Learn about the basic aspects of analytical chemistry.
- Identify the types of chemical analysis.
- To differentiate the qualitative and quantitative analysis.
- Understand the difference between fractional and systematic analysis of cations and anions.
- Understand the group reagents and their proper selection for separation of various cations.
- Know the basic requirements for a suitable group reagent.
- Know about the rare elements, their categorization in various groups and possible abundance along with the various cations.
- To execute the qualitative analysis of mixture of salts containing rare elements.

1.2 INTRODUCTION

Analytical chemistry is a science concerned with development of the theoretical foundations and methods of chemical analysis. It involves separating, identifying and determining the relative amounts of components in a sample of matter. The practical objective of analytical chemistry is to establish the composition of naturally occurring or artificially manufactured substances. The discipline of analytical chemistry consists of qualitative analysis and quantitative analysis. **Qualitative analysis** reveals the chemical identity of the species in the sample and the manner in which the component element or groups of elements are combined with each other. As an example, if a pollutant in a river has been killing fish, qualitative analysis could be used to determine the chemical identity of the pollutant. Qualitative analysis would tell the analyst whether the pollutant was a heavy metal, such as lead, cadmium or mercury, or some other substance. Qualitative analysis is not a method for determining the concentration of a substance. If the identity of the chemical species is known, the concentration of that species can be determined by quantitative analysis.

Quantitative analysis establishes the relative amount of one or more of these species, or analytes, in numerical terms. Qualitative information is required before a quantitative analysis can be undertaken. This is because the most efficient and convenient quantitative method for the determination of a particular analyte or component is chosen when the substances (ions, elements or compounds) present are identified by qualitative analysis. The components of a sample that are to be determined are often referred to as analyte. One of the first things that a chemist must find out about a sample of matter with which he/she is working is the nature of the components. The determination of the species present in a sample of matter is the area of chemistry known as qualitative analysis. Once we know what substances are in a sample we can go further and carry out quantitative analysis to establish how much of each substance is there. In this unit, we will restrict our attention to problems of qualitative analysis and we will discuss fractional and systematic analysis of ions, classifications of cations and anions into groups on the basis of their similarity in reaction towards a group reagent, representative reactions of selected cations and anions, confirmatory tests for some cations and anions, and how to identify cations and anions from complex mixture.

1.3 FRACTIONAL AND SYSTEMATIC ANALYSIS OF IONS

The development of a general procedure to analyze a solution that might contain a mixture of cations or anions would be a formidable problem. One of your tasks in this unit is to be become reasonably well acquainted with one such procedure. Many ions can be detected directly in separate portions of the test solution using specific reactions irrespective of the presence of other ions. The sequence in which individual ions are detected is not important. Such detection or analysis of ions by their specific reactions is known as **fractional analysis**. In the absence of sufficiently specific reactions and when the selectivity can't be increased in any way, ions can't be detected by fractional method. In such cases, one has to plan a definite sequence of reactions for detection of individual ions and this way of detecting ions is known as **systematic analysis**. In this type of analysis, each ion is detected only after all the ions that interfere with its detection (i.e., which react in similar manner with the reagent used) have been detected and removed from solution. The systematic analysis of ion is based upon the successive precipitation of group of ions, so that the total number of ions can be broken down into smaller number of groups, each containing a number of related ions. It means that in systematic analysis, it is necessary to use separation reactions and detection reactions for individual ions. In systematic qualitative analysis, we use the general scheme on the many differences in properties of the ions with respect to a rather small group of common reagents. Even if there is specific reaction for all ions, the systematic analytical scheme retains its value since it is more convenient and economic than fractional analysis of a substance of unknown composition. However, if the composition of a substance is approximately known (as is often actually the case) and it is necessary only to establish the presence or absence of one or two impurities, fractional reactions are more advantageous.

1.4 SYSTEMATIC QUALITATIVE ANALYSIS OF CATIONS

For the purpose of systematic qualitative analysis, cations are classified into six groups on the basis of their behaviour against some reagents. The classification makes the study of reaction of cations easier because ions of analogous behaviour are dealt within one group. In systematic analysis, ions are separated from complex mixtures into groups, advantage being taken of the same reactions of the ions within a group with a reagent called group reagent. The group reagent

helps us to decide the presence or absence of group of ions, and can also separate these groups for further examination. A Group reagent is a specific reagent that precipitates or dissolves one group of ions out of group of ions by forming a slightly soluble compound or precipitates while the ions of other groups remain in solution or form precipitate. The use of group reagent has considerable advantages, as the complex problem of analysis is subdivided into a number of simpler operations. If any group is entirely absent, its group reagent doesn't give any precipitate with the unknown solution. It is not then necessary to carry out test for the individual ion of this group, which saves a considerable amount of work, reagent and time.

A reagent used as group reagent must meet the following requirements:

i.   It must precipitate cations practically quantitatively. That is, it must precipitate cations almost completely; the cation concentration after precipitation should not exceed 10^{-6} mol/liter.

ii. The resulting precipitate must be dissolved in acid (appropriate solvent) so that analysis can be continued.

iii. The excess of the group reagent added must not interfere with the detection or identification of the ions left in solution. For example, for the separation of Group III cations the group reagent is excess NaOH. Excess of the NaOH added to a sample containing mixture of cations for separation of this group ion should not interfere with the detection of Group IV and V cations.

1.5 *QUALITATIVE ANALYSIS OF MIXTURE OF SALTS CONTAINING RARE ELEMENTS*

Elements like tungsten, molybdenum, titanium, thorium, cerium, uranium, beryllium, zirconium, vanadium, selenium, thallium and lithium are regarded as the rare elements; because these elements are less commonly occur in the samples in comparison to the other elements which occur more often in ordinary samples. These rare elements are generally classified in to three groups as given below:

1. First group: Thallium and Tungsten
2. Second group: Selenium, Tellurium and Molybdenum

3. Third group: Titanium, Zirconium, Vanadium, Cerium, Thorium, Uranium, Beryllium and Lithium

The above discussed rare elements are usually given with the mixture containing the common cations (basic radicals). Therefore the identification and separation of these elements/ions must be carried along with the basic radicals present in first three groups. Following procedures is followed for the separation of rare elements from the mixture.

Since the analysis of basic radicals is performed by dissolving the mixture in suitable solvents based on its solubility. Therefore to prepare the solution of basic radicals, first dissolve the mixture in cold water. If it is soluble then go for further analyses. If the mixture is not soluble in cold water then dissolve it in hot water, if it is soluble then go for further analyses. Similarly, depending on the solubility of mixture the solution is prepared in various solvent in the following order: cold water, hot water, dilute HCl, conc. HCl, dil HNO_3, conc. HNO_3 and aquaregia (conc. HNO_3 + conc. HCl:: 1:3).

Removal of interfering radicals: However, in general, take 0.1g of the given mixture in a porcelain dish. To this add nearly 10 drops of conc. HNO_3 and evaporate the contents on a sand bath to almost dryness. When the dried mixture is still moist, add nearly 10 drops of conc. HCl and continue the evaporation on a sand bath till complete dryness. This process is repeated for 2-3 times to remove the interfering radicals Borate (BO_3^-), Fluoride (F^-) and Oxalate ($C_2O_4^{-2}$).

Separation of cations into groups: After the removal of interfering radicals treat the dry mass with 5 mL of hot dilute HCl (2 M). Now the solution thus obtained is used for the analysis of cations which are separated in different groups according to the scheme given below (Table 1.1):

Table 1.1

In the above prepared solution add a few drops of dil. HCl (4M). If the precipitate is obtained add few more drops of dil. HCl (4M) to ensure the complete precipitation. Separate the precipitate either by filtration or by centrifuge.					
Residue: White ppt. **Group I present.** It may contain AgCl, Hg_2Cl_2, $PbCl_2$, TlCl and $WO_3.xH_2O$	**Filtrate**: Heat the solution and saturate it with H_2S for about 2-3 minutes and filter. Dilute the filtrate with 5 mL of water, heat it and again pass H_2S gas to ensure the complete precipitation, filter the precipitate. Collect the residue and filtrate for further analysis.				
	Residue: It may contain sulphides of Hg, Pb, Bi, Cu, Cd, As, Sb, Sn along with Mo, Se, and Te. **Group-II present** together with sulphides of Au, Pt and Pd.	**Filtrate**: Remove the dissolve H_2S gas completely by boiling the filtrate. Add few drops of conc. HNO_3 and boil it again. Cool the solution and add about 1 mL of NH_4Cl followed by dil NH_4OH until the solution becomes ammonical. Add few more drops of NH_4OH, heat the content in water bath for few minutes and filter it.			
		Residue: It may contain $Al(OH)_3$, $Cr(OH)_3$, $Fe(OH)_3$, $Be(OH)_2$, $TiO_2.xH_2O$, $ZrO_2.xH_2O$, $MnO_2.xH_2O$, $ThO_2.xH_2O$, $CeO_2.xH_2O$, $(NH_4)_2U_2O_7$, $V(OH)_3$. **Group III present.**	**Filtrate**: Treat the solution with about 10 drops of dil. NH_4OH and pass H_2S gas in hot solution till complete precipitation. Filter the solution.		
			Residue: It may contain the sulphides of Zn, Mn, Co, and Ni. **Group IV present.**	**Filtrate**: Boil the solution to ensure the complete removal of H_2S gas and evaporate the solution to about 1/3 of its volume. To this solution add 1mL of 20% of NH_4Cl followed by dil NH_4OH to make it ammonical. Add 2 mL of $(NH_4)_2CO_3$ under constant stirring. Warm the solution and filter.	
				Residue: It may contain the $BaCO_3$, $CaCO_3$, $SrCO_3$. **Group V present.**	**Filtrate**: Treat the solution with 1 mL of $(NH_4)_2C_2O_4$ and filter it. To the clean filtrate solution add few drops of Na_2HPO_4 reagent. Warn the solution and scratch the sides of test tube with a glass rod. Formation of white precipitate confirms the presence of **Group VI.**

1.5.1 ANALYSIS OF GROUP I CONTAINING RARE ELEMENTS (W AND Tl):

The white precipitate obtained after the addition of dil HCl in the original solution of the inorganic mixture may contain AgCl, Hg_2Cl_2, $PbCl_2$, TlCl and $WO_3.xH_2O$. Wash the precipitate

with cold water. Boil the precipitate with 10 mL of water and filter it while it is hot. Analysis of I group elements along with the W and Tl may be performed as (Table 1.2):

Table 1.2

Residue: The residue may contain AgCl, Hg$_2$Cl$_2$ and WO$_3$.xH$_2$O. Wash the precipitate several times with hot water until the complete removal of Tl and Pb (interference: no precipitate will form with K$_2$CrO$_4$ when added to washing if Tl and Pb are completely removed).Treat the residue with 5 mL of warm dil NH$_4$OH and filter it:			**Filtrate**: It may contain PbCl$_2$ and TICl. On cooling it get crystallize. Add 2-3 mL conc. H$_2$SO$_4$ and evaporate until fumes are stopped. Cool and dilute woth 10-15 mL of water and filter it:	
Residue: If the residue is black it indicates the presence of mercurous ion. Dissolve the black residue in 1-2 mL of conc. HNO$_3$ and dilute with equal volume of water. Add SnCl$_2$ solution in this, a white ppt turning into grey confirms the presence of Hg$^+$ (mercurous) ion.	**Filtrate**: It may contain [Ag(NH$_3$)$_2$]Cl and ammonical tungstate. Neutralise the ammonia by adding dil. HCl and add just enough NH$_4$OH to redissolve any ppt formed. Add KI solution and filter it:		**Residue**: It may contain the PbSO$_4$. Dissolve the ppt in ammonium acetate solution and divide it into two parts: (i) Add few drops of KI solution. Formation of shining yellow crystals of PbI$_2$ confirms the presence of Pb. (ii) Add few drops of K$_2$CrO$_4$ solution. Formation of yellow ppt of PbCrO$_4$ confirms the presence of Pb.	**Filtrate**: It may contain Tl$_2$SO$_4$. Neutralise the solution with dilute NH4OH solution and add KI solution. Formation of yellow ppt which is insoluble in sodium thiosulphate solution confirms the presence of Tl.
	Residue: If yellow precipitate of AgI is formed, it confirms the presence of Ag.	**Filtrate**: It may contain W (tungsten). Evaporate the filtrate to a small volume. Add Sn pieces and conc. HCl, boil it. If blue ppt or blue coloration appears W is confirmed.		

1.5.2 ANALYSIS OF GROUP II ELEMENTS CONTAINING RARE ELEMENTS (Se, Te AND Mo):

In the filtrate of the first group add 1-2 mL of dil HCl, heat the content and pass H_2S gas in warm solution. Filter the solution. Repeat the above process of (i.e. dilution, heating and passing H_2S gas) with filtrate until no more precipitate is formed. Filter it again and take the whole precipitate for analysis of II group and keep the filtrate for the analysis of III group. Wash the precipitate with a small volume of 5% NH_4Cl that has been saturated with the H_2S and transfer in a porcelain dish. Add 5-10 mL of ammonium polysulphide solution, heat the solution up to 60 °C and maintain at this temperature for 3-4 minutes with constant stirring. Filter the solution.

Residue: The residue may contain HgS, PbS, Bi_2S_3, CuS, CdS, PdS along with traces of sulphides of Au, Pt, Mo and Sn. Group II A present. It is also called the copper group.

Filtrate: The solution may contain the soluble thio salts of As, Sb, Sn, Mo, Se and Te. Acidify the solution by drop wise addition of HCl (test with litmus paper). Formation of a coloured precipitate indicates the presence of Group IIB.

   a) **Analysis of Group II A (The Copper Group):**
Shake the residue with hot water and filter it. Decant off the clear supernatant and transfer the precipitate in a porcelain dish. Add 3 mL of 6M HNO_3 (acid to water ratio 1:2) in it and heat the content to boiling on a sand bath for 2-3 minutes. Filter the solution and proceed for analysis as given procedure (Table 1.3):

Table 1.3

Residue: It may contain HgS, Pt and Au. Boil it with conc. HCl and small amount of Br$_2$ water and then filter it if traces of SnO$_2$ and PbSO$_4$ are present. Add 1-2 mL of KCl solution and 1-2 mL of HCl and concentrate the solution. Filter it.		Filtrate: It may contain the nitrates of Pb, Bi, Cu, Cd and Pd. Take a small part of it and add dil. H$_2$SO$_4$. If white ppt appears then add ethyl alcohol and dil. H$_2$SO$_4$ to whole solution and filter it.		
Residue: Yellow crystalline ppt of K$_2$[Pt(Cl)$_6$] confirms the presence of Pt.	**Filtrate:** The filtrate may contain HgCl$_2$ ans AuCl$_3$. Boil the solution to remove the excess of the acid, make it alkaline with NaOH solution and boil again with excess of oxalic acid and filter the solution. **Residue:** It the residue is brownish black or purplish black. It confirms the presence of	**Residue:** White ppt of PbSO$_4$. Dissolve the ppt in ammonium acetate and acidify with acetic acid and add K$_2$CrO$_4$ solution. Bright yellow ppt of PbCrO$_4$ confirms the presence of Pb.	**Filtrate:** It may contain sulphates of Bi, Cu, Cd and Pd. Add excess of NH$_4$OH and filter the solution:	
			Residue: White ppt of Bi(OH)$_3$. Wash it with water and dissolve it in minimum amount of dil. HCl. Divide the solution in two part: (i) To the first part add excess of water. White ppt or turbidity of BiOCl confirms the Bi. (ii) To the second part add sod. Stannite solution. Black ppt of finally divided Bi metal confirms the Bi.	**Filtrate:** It may contain [Cu(NH$_3$)$_4$]$^{+2}$ (blue colored) and [Cd(NH$_3$)$_4$]$^{+2}$ (colourless) along with Pd^{+2}. If the solution is colourless Cu^{+2} is absent. Pass H$_2$S gas, if yellow ppt of CdS is obtained. If the solution is blue coloured, then (i) Acidify one part of the filtrate with dil acetic acid and add K$_4$[Fe(CN)$_6$] solution. If reddish brown ppt of Cu$_2$[Fe(CN)$_6$] is formed. Cu is confirmed. (ii) Treat another part of filtrate with 1 g of solid sodium bicarbonate and boil the content on asand bath for 1-2 minutes and filter it:
			Residue: It may be CdCO$_3$, wash it with hot water, dissolve in dil HCl and pass gas, if yellow ppt of CdS confirms Cd.	**Filtrate**: It may be [Cu(NH$_3$)$_4$]$^{+2}$, Acidify it with dil acetic acid and add K$_4$[Fe(CN)$_6$] solution. If reddish brown ppt of Cu$_2$[Fe(CN)$_6$] is formed. Cu is confirmed.

Au. **Filtrate:** It may contain $HgCl_2$. Add few drops of $SnCl_2$ solution. A white ppt turning to grey indicates the presence of Hg.				(iii) After separation of Cu and Cd acidify the solution with dil HCl and add few pieces of granulated zinc. Allow the solution to stand for some time and then filter. Wash the residue with water and 2mL aqua regia. Evaporate about to dryness and dissolve the dry mass to 2 M HCl and add dimethyl glyoxime reagent. Yellow ppt of palladium dimethyl glyoxime $Pd(C_4H_7O_2N_2)_2$ confirms the presence of Pd.

b) Analysis of Group IIB:

The precipitate of Group B may contain the sulphides of As, Sb, Sn, Se, Mo and Te. Treat the ppt with 5 mL of conc. HCl in a small conical flask and boil for 5 minutes. Dilute the content with 2-3 mL of water and filter it, and analyze the elements as given procedure (Table 1.4):

Table 1.4

Residue: The residue may contain sulphides of As, Mo, Se and Te. Dissolve the ppt in conc. HCl and add a small amount of $KClO_3$. Make it alkaline and add $Mg(NO_3)_2$ reagent or magnesia mixture. Shake the solution and stand for 5 minutes, then filter the content.			**Filtrate**: It may contain Sb and Sn as their chlorides or complex chloro salts ($HSbCl_4$ and H_2SnCl_6). Boil off H_2S completely and divide the solution in to two parts:	
Residue: White crystalline ppt of $Mg(NH_4)AsO_4$. $6H_2O$. As is confirmed	**Filtrate:** It may contain Mo, Se and Te as their chlorides or chloro acids. Boil to remove ammonia, add oxalic acid and boil it again for few minutes. Add conc. HCl and concentrate in a water bath. After removing the precipitate KCl, treat the content with slight excess of solid Na_2SO_3 and filter it. **Red residue** confirms the presence of Se.			
	Filtrate: Dilute the filtrate with equal amount of water, add small amount of KI solution and excess of solid Na_2SO_3 and filter it:		**Ist Part:** Make it alkaline with NH_4OH solution. Add 2g of solid oxalic acid and pass H_2S. Filter the solution. Orange ppt of Sb_2S_3 confirms the presence of Sb. To the filtrate add NH_4OH to make it alkaline and pass H_2S. Dirty yellow ppt formation of SnS_2 confirms the	**IInd Part:** Treat it with iron nail, warm it for few minutes and filter it. Black residue confirms the presence of Sb. Add $HgCl_2$ solution to the clear filtrate. A whitish grey ppt formation confirms the
	Residue: Black ppt confirms the	**Filtrate:** Boil with HCl to remove dissolve SO_2 and treat successively with 10% NH_4SCN solution and small amount of $SnCl_2$		

	presence of Te.	solution. Red coloration confirms the presence of Mo.	presence of Sn.	presence of Sn.

1.5.3 ANALYSIS OF GROUP III ALONG WITH Ti, Zr, V, Ce, Th, U, Be AND Li:

Boil off dissolved H_2S completely from the filtrate obtained from II group, add 2-3 drops of conc. HNO_3 and boil again. If any of the interfering radicals (borate, oxalate, fluoride and phosphate) have been detected, they must be removed at this stage. Add excess of NH_4Cl solution and NH_4OH to this. Heat the solution and filter it. The residue thus obtained may contain the hydroxide of group III metals. The filtrate should be kept for the analysis of group IV and subsequent group radicals. Analysis of these radicals may be performed as Table 1.5.

Table 1.5

Dissolve the ppt in minimum volume of dilute HCl. Mix this weakly acidic solution with an equal volume of solution containing 6% H_2O_2 and 10% NaOH. Boil the content for few minutes and filter it.		
Residue: It may contain $Fe(OH)_3$, $Be(OH)_2$, $TiO_2.nH_2O$, $ZrO_2.nH_2O$, $ThO_2.nH_2O$, $CeO_2.nH_2O$ and small amount of $MnO_2.nH_2O$. Dissolve the residue in dil. HCl and boil to expel Cl_2 gas and divide the solution in to 5 parts. i) To the first part add NH_4SCN solution. If red colour is obtained, **Fe is confirmed**. ii) If iron is present add sufficient amount of H_3PO_4 to decolorize ferric salt and then add H_2O_2. If orange red colour is obtained which disappears by the addition of solid NH_4F or NaF, **it confirms the presence of Ti.** iii) To another part add little amount of H_2O_2 and sodium phosphate solution. If white ppt is obtained, it **confirms the presence of Zr.**	**Filtrate**: It may contain Na_2CrO_4, $NaAlO_2$, Na_3VO_4 and Na_2UO_4. Acidify the solution with dil. HNO_3. Add 3-4 mL of 20% $Pb(NO_3)_2$ followed by 2g solid ammonium acetate. Shake it well and filter:	
	Residue: It may contain $PbCrO_4$ and $Pb(VO_3)_2$. Wash the ppt with hot water and dissolve it in minimum quantity of dil and hot HNO_3. Cool the solution and add equal amount	**Filtrate:** It may contain $Al(NO_3)_3$ and $UO_2(NO_3)_3$ along with the excess of Pb^{+2}. Pass H_2S to remove Pb as PbS completely. Boil off H_2S from the filtrate. Neutralize the solution with NH_4OH and pour into an excess of concentrated $(NH_4)_2CO_3$ solution. Warm it and allow it to stand for some

iv) To another part add excess of saturated oxalic acid solution and filter the content.		volume of amyl alcohol and a little quantity of 6% H_2O_2. Allow the solution to stand for some time. Two layers will be formed. A blue colouration in the upper layer confirms the **presence of Cr**. A red brownish colouration in the lower layer confirms the **presence of V.**	time. Filter it:	
Residue: If it is white, treat with conc. $(NH_4)_2C_2O_4$ solution and filter it.				
Residue: It is cerium oxalate. **Ce is confirmed.**	**Filtrate:** If white ppt is obtained on addition of dil. HCl. It **confirms the presence of Th.**	**Filtrate:** Add H_2O_2 and NaOH, if cold white gelatinous ppt of $Be(OH)_2$ forms and it dissolves in excess of NaOH forming Na_2BeO_2. It **confirms the presence of Be**.	**Residue:** White ppt of $Al(OH)_3$ confirms the presence of Al.	**Filtrate:** It may contain $(NH_4)_2[UO_2(CO_3)_2]$. Evaporate to small volume and add 2-3 drop of dil HCl and $K_4[Fe(CN)_6]$ solution. Brown ppt of $(UO_2)_2[Fe(CN)_6]$ which turns yellow to adding NaOH confirms the **presence of Uranium, U.**
v) Evaporate the last portion with H_2SO_4 till the fumes stop. If HCl is completely removed, cool the sotution and add conc. HNO_3 and PbO_2 and heat the content. Formation of pink colouration **confirms the presence of Mn**.				

1.5.4 ANALYSIS OF GROUP IV CATIONS:

To the filtrate from group III, add about 2 mL of NH_4OH, pass H_2S gas till the complete precipitation. Filter the content and keep the filtrate for the analysis of Vth group. Wash the residue, which contain sulphides of Zn, Co, Ni and Mn, with hot water and add 4mL of 2M HCl (1 part of HCl and 5 parts of water). Stir the solution for couple of minutes and filter the content. Analyze the respective elements as per given procedure in Table 1.6.

Table 1.6

Residue: Black residue indicates the presence of NiS and CoS. Dissolve the ppt in hot aquaregia. Evaporate the solution till dryness and extract with water. Divide the content into three parts. (i) In the first part add 3-4 drops of Dimethyl glyoxime solution and a small amount of NH_4OH (till the appearance of ammonia like smell). The formation of pink ppt **confirms the presence of Ni.** (ii) To the second part add dil. CH_3COOH and 0.1 g of solid KNO_2. The formation of yellow ppt of $K_3[Co(NO_2)_6]$ **confirms the presence of Co**. If $NaNO_2$ is used instead of KNO_2 yellow colored solution is obtained. It also **confirms the presence of Co.** (iii) In the third part add NH_4SCN and amyl alcohol. The alcohol layer turns blue due to the presence of a complex species of Co. It also **confirms the presence of Co.**	**Filtrate**: It may contain $MnCl_2$ and $ZnCl_2$. Boil off the H_2S gas completely. Cool the solution and add 4 mL of NaOH. Warm the solution and on water bath for about 2 minutes and filter it:
	Residue: It may be $Mn(OH)_2$. Treat the residue with 2 mL of conc. HNO_3 and add a pinch of PbO_2. Warm the content and filter it. A pink colored supernate **confirms the presence of Mn.**
	Filtrate: It may contain Na_2ZnO_2. Pass H_2S gas in the filtrate. Formation of a dirty white ppt of ZnS **confirms the presence of Zn.**

1.5.5 ANALYSIS OF GROUP V CATIONS:

Boil off the dissolved H_2S gas completely from the filtrate of IVth group. Evaporate it to its half of the volume. Cool the solution and add 1 mL of 20% NH_4Cl followed by dil NH_4OH to make it ammonical. Now add 2 mL of $(NH_4)_2CO_3$ solution under constant stirring. Warm the solution and filter it. The filtrate is kept for the test of Mg^{+2} i.e. Group VI.

The residue thus obtained may contain Ca, Sr and Ba. Wash the residue with hot water and reject the washing. Treat it with dil CH_3COOH drop by drop until the ppt is completely dissolved. Warm the content on a water bath and add 1-2 mL of K_2CrO_4 solution in whole content and filter it. Analyze the respective elements as per given procedure in Table 1.7.

Table 1.7

Residue: Yellow residue of BaCrO$_4$ gives an indication for the presence of Ba. Apply flame test with ppt. A grassy green flame **confirms the presence of Ba.**	**Filtrate**: It may contain Sr^{+2} and Ca^{+2}. Warm the content and add 1 mL of saturated (NH$_4$)$_2$SO$_4$. Allow to stand it for 5 minutes and filter it:	
	Residue: White residue of SrSO$_4$ indicates the presence of Sr. Apply flame test to the ppt. A Crimson red colored flame formation **confirms the presence of Sr.**	**Filtrate:** It may contain the Ca^{+2} ions. Add 1 mL of (NH$_4$)$_2$C$_2$O$_4$ solution and warm it. Formation of white ppt of Ca C$_2$O$_4$ confirms the presence of Ca. Apply flame test on ppt. Brick red flame formation **confirms the presence of Ca.**

Remember: If excess fo Ca^{+2} ions are present, a white ppt of CaSO$_4$ may be obtained on addition of (NH$_4$)$_2$SO$_4$ even in the absence of Sr^{+2}. In order to ensure the presence of Sr^{+2} few drops of earlier filtrate is treated separately with a drop of saturated CaSO$_4$ solution. The formation of white ppt will confirm the presence of Sr^{+2}. If no ppt is obtained Sr^{+2} is absent. The remaining filtrate is now treated with (NH$_4$)$_2$SO$_4$ as above. Filter the solution and test the filtrate for the analysis of Ca^{+2} ions.

1.5.6 ANALYSIS OF GROUP VI (TEST OF Mg):

Treat the filtrate obtained from the group Vth with 1 mL of (NH$_4$)$_2$C$_2$O$_4$ and filter the content. Reject the residue if any. To the clean filtrate add few drops of Na$_2$PO$_4$. Warm the content and scratch the side wall of test tube with glass rod. White crystalline ppt of MgNH$_4$PO$_4$.6H$_2$O confirms the presence of Mg.

Dissolve this ppt in few drops of dil HCl and then treat the solution with a drop of Magneson II reagent followed by a drop of 10% NaOH solution. Formation of a blue lake confirms the presence of Mg.

1.6 SUMMARY

The above unit comprises a systematic separation procedures of various cations present in a given mixture of salts. The overall unit may be summarized as follow:

• Analytical chemistry is a science concerned with development of the theoretical foundations and methods of chemical analysis. It involves separating, identifying and determining the relative amounts of components in a sample of matter.

• Qualitative analysis reveals the chemical identity of the species in the sample and the manner in which the component element or groups of elements are combined with each other.

- Quantitative analysis establishes the relative amount of one or more of these species, or analytes, in numerical terms.

- Qualitative analysis of a given unknown mixture must perform via systematic analysis.

- Tungsten, molybdenum, titanium, thorium, cerium, uranium, beryllium, zirconium, vanadium, selenium, thallium and lithium are regarded as the rare elements.

- Interfering radicals must removed well before the systematic identification of the cations.

- Prior to the separation of particular group radicals, the cations present in to salt mixture first properly divided in to their respective groups.

UNIT 2: INORGANIC PREPARATIONS

CONTENTS:

2.1 OBJECTIVES

In this unit learner will be able to

- Learn about the laboratory preparation methods of various inorganic compounds specially the coordination complex.

- Understand how to write the laboratory reports in a proper sequential manner.

- Know the structure and hybridization of the various coordination complexes prepared.

- Learn about the magnetic properties of the prepared complex

- Describe the structure of the prepared complex.

- Understand how the ligands are distributed in space around the central metal ion.

2.2 PREPARATION OF BIS (DIMETHYLGLYOXAMATO) NICKEL (II); [Ni(dmg)$_2$] OR [Ni(C$_4$H$_7$O$_2$N$_2$)$_2$]

THEORY:

The bis (dimethylglyoxamato) nickel (II) complex i.e. [Ni(dmg)$_2$] or [Ni(C$_4$H$_7$O$_2$N$_2$)$_2$] is prepared by the reaction of neutral or slightly acidic nickel ammonium sulphate {NiSO$_4$.(NH$_4$)$_2$SO$_4$.6H$_2$O} solution with alcoholic dimethyl glyoxime solution followed by the addition a slight excess of ammonium hydroxide NH$_4$OH. The general reaction of the formation of [Ni(dmg)$_2$] complex is shown as follow
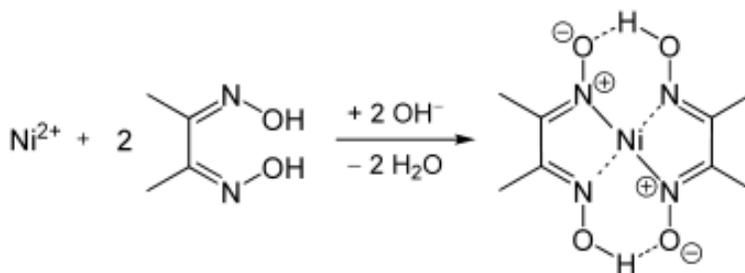
GLASSWARE AND CHEMICALS REQUIRED:

Glassware and apparatus: Beaker, funnel, glass rod, sintered glass crucible or porcelain filtering crucible, conical flask, Water bath Thermometer.

Reagents and Chemicals: Ammonium nickel sulphate {NiSO$_4$.(NH$_4$)$_2$SO$_4$.6H$_2$O}, Alcoholic dimthyl glyoxime (C$_4$H$_7$O$_2$N$_2$), Ammonium hydroxide NH$_4$OH, Dilute HCl.

PROCEDURE:

Weigh out accurately 0.3-0.4g of pure nickel ammonium sulphate and dissolve it in 10 mL of water. Add 5mL of dil. HCl in it and dilute the resulting solution to 200mL. Heat the solution to 70-80°C, add a slight excess (about 50 mL) of alcoholic dimethyl glyoxime reagent. Immediately add dilute ammonia solution (NH$_4$OH) dropwise with stirring until precipitation occurs, then add 15-20 drops excess of NH$_4$OH solution to ensure the complete precipitation. Heat the solution on water bath about 15-20 minutes and allow it to cool at laboratory temperature. Formation of red coloured precipitate indicates the completion of the reaction. Allow the precipitate to stand for 15 minutes. Filter the precipitate through a pre-weighed sintered glass crucible or porcelain

filtering crucible. Wash the precipitate with cold water until free from chloride and dry it at 110-120°C for about 30 min. Allow to cool in a desiccator, weigh and report the yield.

REACTIONS INVOLVED:

Following reactions are involved in the formation of bis (dimethylglyoxamato) nickel (II) complex.

$$NiSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O \longrightarrow NiSO_4 + (NH_4)_2SO_4 + 6H_2O$$

$$NiSO_4 + 2 \quad \text{(dimethylglyoxime)} + NH_4OH \longrightarrow Ni(C_4H_7O_2N_2)_2 + (NH_4)_2SO_4 + 2H_2O$$
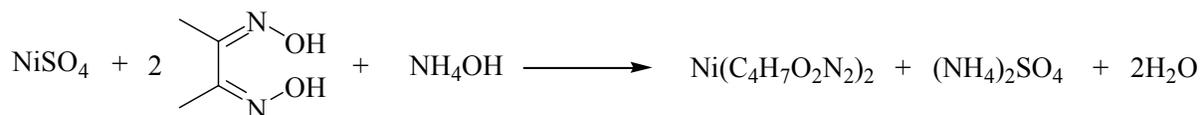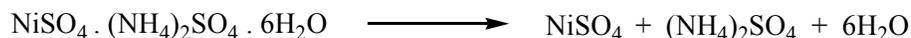
RESULTS:

(i)   Physical state: Solid
(ii) Colour: Red
(iii) Yield: ……… g

STRUCTURE:

Two molecules of bidentate dimethyl glyoxime ligand are attached with Ni (II) in a square planar geometry involving dsp^2 hybridization. It is diamagnetic in nature. The coordination number of Ni is 4. The structure of bis (dimethylglyoxamato) nickel (II) complex is shown as follow.
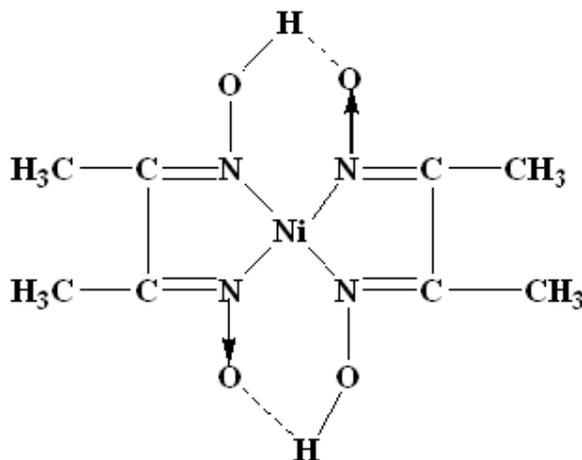
Structure of bis (dimethylglyoxamato) nickel (II) complex

2.3 PREPARATION OF PRUSSIAN BLUE i.e. Fe$_4$[Fe(CN)$_6$]$_3$:

THEORY:

Prussian blue is a dark blue pigment with the formula Fe$_4$[Fe(CN)$_6$]$_3$. Another name for it is Berlin Blue, or sometimes in painting, Parisian Blue. It was one of the first synthetic pigments used by humans. It is employed as a very fine colloidal dispersion, as the compound itself is not soluble in water. In medicine, Prussian blue is used as an antidote for certain kinds of heavy metal poisoning, e.g., by cesium and thallium. In particular it was used to absorb ^{137}Cs$^+$ from those poisoned in the Goiania accident. Prussian Blue is orally administered. The therapy exploits Prussian Blue's ion exchange properties and high affinity for certain "soft" metal cations. Prussian blue is produced by oxidation of ferrous ferrocyanide salts. It contains ferric hexacyanoferrate(II) in a cubic lattice crystal structure. It is insoluble in water but also tends to form a colloid thus can exist in either colloidal or water-soluble form, and an insoluble form. Prussian blue is included in the World Health Organization Model List of Essential Medicines as a specific antidote used in poisonings to provide symptomatic and supportive treatment. The structure of Prussian blue is shown as:
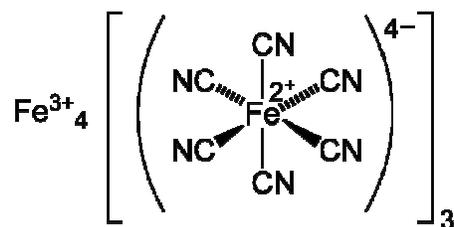
GLASSWARE AND CHEMICALS REQUIRED:

Glassware and apparatus: Beaker, Buchner funnel, filter flask, tubing, filter-vac rubber rings, conical flask, filter paper.

Reagents and Chemicals: Iron fillings, Potassium Ferrocyanide (K$_4$[Fe(CN)$_6$]), Sulphuric acid.

PROCEDURE:

Take 2-3 g of iron fillings in a 250 mL of conical flask. To this add 50 mL of dilute H$_2$SO$_4$; close the flask with rubber cork carrying long glass tubing. Heat the solution gently on a low flame for 5-10 minute. Cool it at laboratory temperature and then filter it. Preserve the filtrate and transfer the precipitate back to the same conical flask. To this add another 25 mL of dilute H$_2$SO$_4$; close

the flask with rubber cork carrying long glass tubing. Again, heat the solution gently on a low flame for 5-10 minute, cool it and then filter it. Mix both the filtrate in a 500 mL beaker and add saturated solution of $K_4[Fe(CN)_6]$ (Potassium Ferrocyanide) to it. Heat the resultant solution until it turns to green and then transfer this green solution in a porcelain dish. Allow the solution to get oxidized in open air until the green colour changes to blue (usually oxidation in presence of air for 3-4 hrs). The oxidation process may become fast if 2-3 mL of dil $K_2Cr_2O_7$ is added in it. Filter the blue precipitate and wash it with ethanol and dry it. Take its weight and report the yield.

REACTIONS INVOLVED:

Following reactions are involved in the formation of Prussian blue pigment ($Fe_4[Fe(CN)_6]_3$).

$$2Fe + 3H_2SO_4 \longrightarrow Fe_2(SO_4)_3 + 3H_2$$

$$2Fe_2(SO_4)_3 + 3K_4[Fe(CN)_6] \longrightarrow Fe_4(Fe(CN)_6)_3 + 6K_2SO_4$$
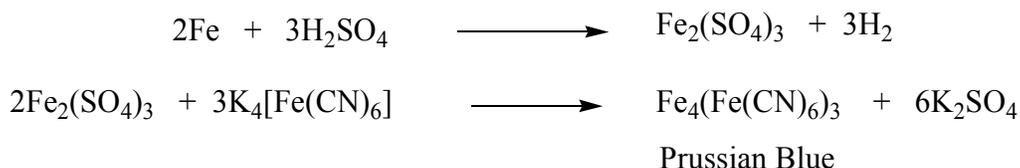
Prussian Blue

RESULTS:

(i) Physical state: Solid
(ii) Colour: Blue
(iii) Yield: ……… g

STRUCTURE:

The structure of Prussian blue is shown in given figure. Four Iron (III) ions are surrounded by three hexacyanoferrate (II) ions.
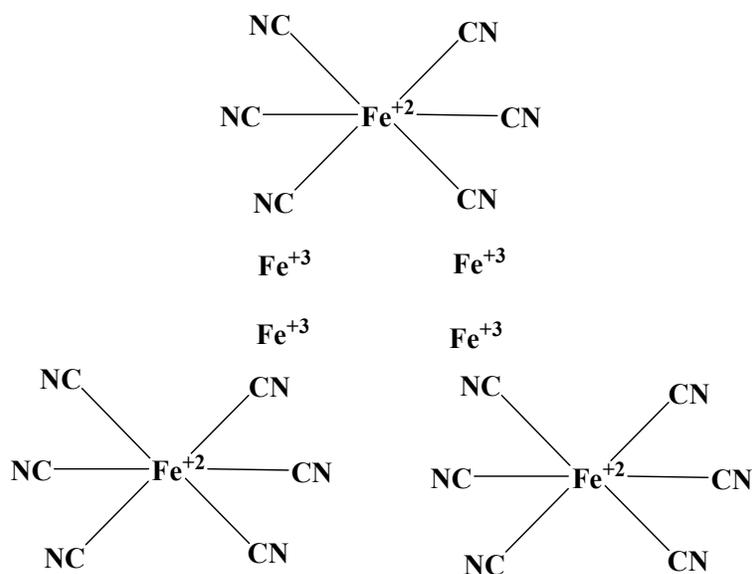
Structure of Prussian blue complex

2.4 PREPARATION OF TURNBULL'S BLUE i.e. Fe₃[Fe(CN)₆]₂:

The procedure for the preparation of Turnbull's blue is quite similar to that of Prussian blue. It involves the reaction of Fe(II) with $K_3[Fe(CN)_6]$ to produce compound known as iron(II) hexacyanoferrate (III) i.e. $Fe_3[Fe(CN)_6]_2$. This product was traditionally named Turnbull's blue (TB). The Turnbull's blue is having fade colour in comparison to the Prussian blue. However X-ray diffraction and electron diffraction methods have shown, though, that the structures of Prussian Blue and Turnbull's Blue are identical. The differences in the colours for Prussian Blue and Turnbull's Blue reflect slight differences in the methods of precipitation, which strongly affect particle size and impurity content.

In its preparative reactions, the hexacyanoferrate (III) ion oxidises Fe (II) to Fe (III) and itself get reduced to hexacyanoferrate (II)

$$Fe^{+2} \ + \ [Fe(CN)_6]^{-4} \longrightarrow Fe^{+3} \ + \ [Fe(CN)_6]^{-4}$$

The Fe (III) ion reacts with $[Fe(CN)_6]^{4-}$ to produce Prussian blue.

$$4Fe^{+3} \ + \ 3[Fe(CN)_6]^{-4} \longrightarrow Fe_4(Fe(CN)_6)_3$$
$$\text{Prussian Blue}$$
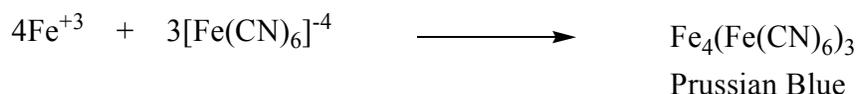
The less intense blue colour in case of Turnbull's blue is due to the formation of a white compound (actually light green) having formula $Fe_3[Fe(CN)_6]_2$ *i.e.* ferrous ferrocyanide. The ferrous ferrocyanide is formed by the reaction of Fe(II) salt added initially to $K_3[Fe(CN)_6]$ solution.

$$3FeSO_4 + 2K_3[Fe(CN)_6] \longrightarrow \underset{\text{Turnbull's Blue}}{Fe_3(Fe(CN)_6)_2} + 3K_2SO_4$$
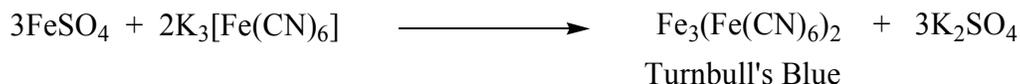
Although during the reaction most of the compound is readily oxidises to Prussian blue but some of the unoxidised form of the compound may be responsible for the fade colour of Turnbull's blue. It can also be stated that during the preparation of the Prussian blue if some of the ferrous ferrocyanide is not oxidized to ferric ferrocyanide (Prussian blue) the resulting product thus obtained would be less intense in comparison to Prussian blue. This compound is termed as Turnbull's blue.

Note: The Chemical and glassware requirement for the preparation are quite similar. However, $K_3[Fe(CN)_6]$ is used instead of $K_4[Fe(CN)_6]$.

2.5 PREPARATION OF TETRAAMMINE COPPER (II) SULPHATE MONOHYDRATE i.e. $[Cu(NH_3)_4]SO_4.H_2O$:

THEORY:

Tetraammine copper (II) sulphate is an inorganic coordination compound with the formula $[Cu(NH_3)_4]SO_4.H_2O$. This dark blue solid is a metal complex with faint odour of ammonia. It is closely related to Schweizer's reagent, which is used for the production of cellulose fibers in the production of rayon. It is used to print fabrics, used as a pesticide and to make other copper compounds like copper nano-powder. The Tetraammine copper (II) sulphate complex is highly soluble in water and tends to hydrolyse and liberate ammonia upon air exposure. The brilliant dark blue-violet colour of tetraammine copper (II) sulphate solution is due to presence of $[Cu(NH_3)_4]^{2+}$.

When concentrated ammonia solution is added to a solution of Copper (II) sulphate, a blue coloured precipitate of basic Copper (II) sulphate is first formed. The resulting precipitate is then dissolved in excess ammonia solution which produces an intense blue solution. This intense blue

solution is due to the formation of tetraammine copper (II) sulphate complex. Addition of ethanol to this solution results in the precipitation of deep blue coloured tetraammine copper (II) sulphate complex *i.e.* $[Cu(NH_3)_4]SO_4.H_2O$.

GLASSWARE AND CHEMICALS REQUIRED:

Glassware and apparatus: Beakers, Buchner funnel, filter flask, tubing, filter-vac rubber rings, conical flask, filter paper, glass rod.

Reagents and Chemicals: Copper sulphate ($CuSO_4.5H_2O$), concentrated ammonia solution (NH_4OH), Ethanol.

PROCEDURE:

Take 5 g of finely powdered copper sulphate ($CuSO_4.2H_2O$) in minimum amount of water. To this solution add 10 mL of concentrated ammonia solution (NH_4OH) in it under constant stirring from a dropping funnel until the formation of blue precipitate. Dissolve the precipitate thus formed in excess ammonia by adding 20 mL of ammonia solution. It gives clear blue solution with a distinct smell of ammonia. Now add slowly 30-35 mL of ethanol to the resulting clear blue solution under constant stirring. Cover the beaker with watch glass and allow the solution to stand for 2-3 hrs for crystallization. Filter the blue coloured crystals on Buchner funnel, wash with ethanol and dry in air and report the yield.

REACTIONS INVOLVED:

Following reactions are involved in the formation of tetraammine copper (II) sulphate complex *i.e.* $[Cu(NH_3)_4]SO_4.H_2O$

$$2CuSO_4 + 2NH_3 + 2H_2O \longrightarrow Cu(OH)_2 . CuSO_4 (s) + 2NH_4^+$$

$$Cu(OH)_2 . CuSO_4 (s) + 8NH_3 \longrightarrow 2[Cu(NH_3)_4]^+ (aq) + 2OH^- + SO_4^{-2} (aq)$$

$$2[Cu(NH_3)_4]^+ (aq) + SO_4^{-2} (aq) + H_2O \longrightarrow [Cu(NH_3)_4]SO_4 . H_2O$$
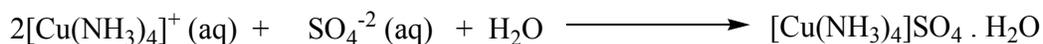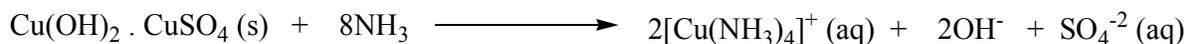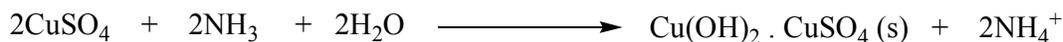
RESULTS:

(i) Physical state: Solid

(ii) Colour: Blue

(iii) Yield: ……… g

STRUCTURE:

The solid state salt of tetraammine copper (II) sulphate contains the $[Cu(NH_3)_4]^{2+}$. Central metal ion *i.e.* Cu(II), bonded with 4 NH_3 molecules using *dsp²* hybridization, therefore the complex has a square planar molecular geometry. The complex has one unpaired electron hence it is paramagnetic in nature. The structure of tetraammine copper (II) sulphate is shown as:
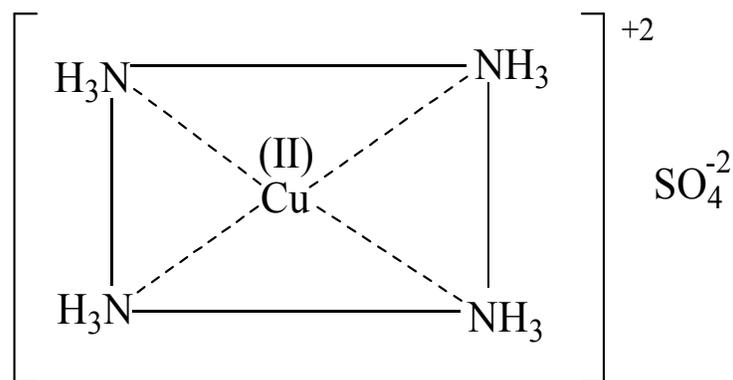
Structure of tetraammine copper (II) sulphate ($[Cu(NH_3)_4]SO_4$)

2.6 PREPARATION OF HEXAAMMINE COBALT (III) HEXANITRO COBALTATE (III) i.e. Co[NH₃)₆][Co(NO₂)₆]:

THEORY:

Hexaamminecobalt(III) chloride is a chemical compound with the formula $[Co(NH_3)_6]Cl_3$. This coordination compound is considered as a Werner complex, named after the pioneer of coordination chemistry, Alfred Werner. This salt consists of $[Co(NH_3)_6]^{3+}$ cations, each accompanied by three Cl⁻ anions. The cation itself is a metal ammine complex with six ammonia molecules as ligands on the cobalt atom. Originally this compound was described as the luteo (Latin: yellow) complex of cobalt, but this name has been discarded as modern chemistry less preferred colour based nomenclature over molecular structure.

GLASSWARE AND CHEMICALS REQUIRED:

Glassware and apparatus: Beakers, Buchner funnel, filter flask, tubing, filter-vac rubber rings, conical flask, filter paper, glass rod, vacuum desiccator, water bath, ice bath.

Reagents and Chemicals: Hexaammine cobalt (III) chloride *i.e.* ($[Co(NH_3)_6]Cl_3$), Hexanitro cobaltate (III) *i.e.* $[Co(NO_2)_6]^{-3}$.

PROCEDURE:

In a 100 mL beaker, take 2 g of Hexaammine cobalt (III) chloride and dissolve it in minimum amount of hot water. In another 100 mL beaker take 3 g of Hexanitro cobaltate (III) and dissolve it in minimum amount of hot water. Add this solution drop by drop to the hot and stirred solution of Hexaammine cobalt (III) chloride. Cool the resulting solution in an ice bath till the complete precipitation. Filter the precipitate with the help of Buchner funnel and dry it in vacuum desiccator. Weigh the dried solid compound and report the yield.

RESULTS:

(i)   Physical state: Solid

(ii) Colour: ………

(iii) Yield: …… g

STRUCTURE:

The Co(III) ions are octahedrally surrounded by 6 ammonia (NH_3) in $[Co(NH_3)_6]Cl_3$ and 6 Nitrito (NO_2^{-2}) in $[Co(NO_2)_6]^{-3}$ complex. Each Cobalt (III) ion is d^2sp^3 hybridized. Whole complex is diamagnetic.
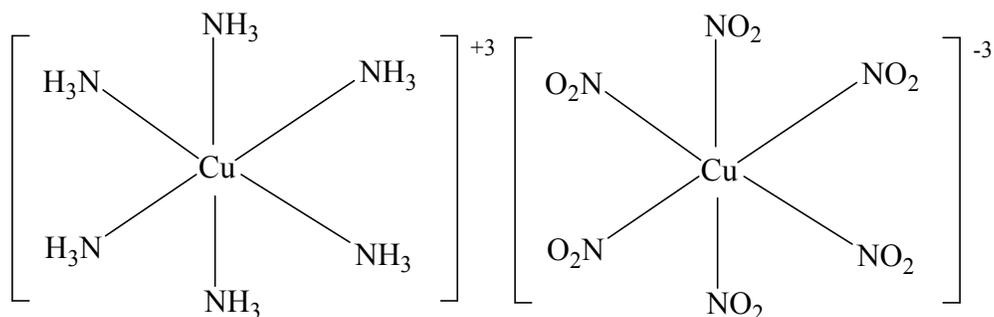
2.7 PREPARATION OF cis-POTASSIUM DIOXALATO DIAQUA CHROMATE (III) i.e.cis-K[Cr(C₂O₄)₂(H₂O)₂]

THEORY:

cis-Potassium dioxalatodiaqua chromate (III) complex is a chemical compound with molecular formula *cis*-K[Cr(C$_2$O$_4$)$_2$(H$_2$O)$_2$]. It is a crystalline material. It is prepared by the reaction of potassium dichromate and oxalic acid.

GLASSWARE AND CHEMICALS REQUIRED:

Glassware and apparatus: Beakers, Buchner funnel, filter flask, mortar-pastel, conical flask, filter paper, glass rod, vacuum desiccator, water bath.

Reagents and Chemicals: Oxalic acid (HOOC-COOH), Potassium dichromate (K$_2$Cr$_2$O$_7$), Ethanol (C$_2$H$_5$OH).

PROCEDURE:

Take finely powdered mixture of 4 g of oxalic acid dihydrate and 1-1.5 g of potassium dichromate in a dry mortar. Mix the powders as intimately as possible by gentle grinding in the mortar. Transfer the mixture in a wet large evaporating dish (~10 cm); the mixture will become moistened by the water that remains in the evaporating dish. Cover the evaporating dish with a large watch glass and warm it gently on a hot plate. A vigorous spontaneous reaction will take place with the release of CO$_2$ gas. The mixture should then liquefy, it resulted deep-colored syrup. Add about 20 mL of 95% ethanol on it and continue to gently warm it on the hot plate. Grind or crush the product with a spatula until it solidifies. If complete solidification cannot be completed with one portion of 95% alcohol, decant the liquid, add another 20 mL of 95% alcohol, warm gently, and again the keep on grinding the mixture till the product is entirely crystalline and granular. Filter the crystals on Buchner funnel wash with ethanol, dry, weigh and report the yield. The crystals look almost black in diffuse daylight and deep purple in artificial light.

REACTIONS INVOLVED:

Following reaction is involved in the preparation of *cis*-Potassium dioxalatodiaqua chromate (III) complex.

$$K_2Cr_2O_7 + 7H_2C_2O_4 \cdot 2H_2O \longrightarrow 2K[Cr(C_2O_4)_2(H_2O)_2] \cdot 2H_2O + 6CO_2 + 13H_2O$$

RESULTS:

(i)   Physical state: Solid

(ii) Colour: ………

(iii) Yield: ……… g

STRUCTURE:

The coordination number of central metal ion Cr in *cis*-Potassium dioxalatodiaqua chromate (III) complex is 6. Hence, it has an octahedral geometry with d^2sp^3 hybridization. The complex is paramagnetic in nature because of the presence of 3 unpaired electrons.
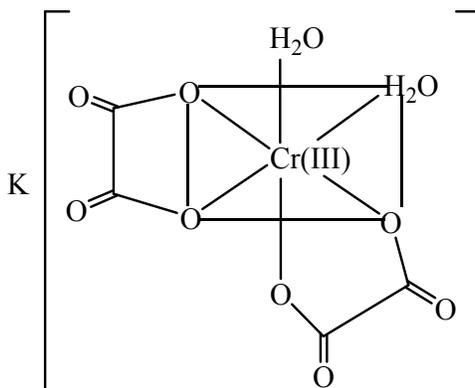
Structure of *cis*-Potassium dioxalatodiaqua chromate (III) complex

2.8   PREPARATION   OF   MERCURY   TETRATHIOCYANATO COBALTATE (II) i.e. Hg [Co(SCN)₄]

THEORY:

Acid base reactions are frequently used to form a variety of salts and coordination complexes. In present experiment, the mercury tetrathiocyanato cobaltate (II) complex is formed according to the hard soft acid base (HSAB) principle. In mercury tetrathiocyanato cobaltate (II) complex,

the Co^{2+} (hard acid) and Hg^{2+} (soft acid) is complexed to SCN^-, a bidentate ligand which is capable of acting as both a hard and soft base.

GLASSWARE AND CHEMICALS REQUIRED:

Glassware and apparatus: Beakers, Buchner funnel, filter flask, mortar-pastel, conical flask, filter paper, glass rod, vacuum desiccator, water bath, oven.

Reagents and Chemicals: Cobalt nitrate {$Co(NO_3)_2.6H_2O$}, Ammonium thiocyanate (NH_4SCN), Mercuric chloride ($HgCl_2$).

PROCEDURE:

In a beaker take 5 g of cobalt nitrate hexahydrate and 6 g of ammonium thiocyanate and dissolve it in 25 mL of boiling water (solution A). In another beaker dissolve 5 g of $HgCl_2$ and dissolve it in 50 mL of water (solution B). Mix the previously prepared solution (solution A) with the solution of $HgCl_2$. Boil the resultant solution in a water bath for 10 minutes. Allow the boiled solution to cool at laboratory temperature. A blue coloured precipitate is formed. Decant the supernatant liquid and wash it with cold water. Filter the precipitate with Buchner funnel and collect the deep blue crystals. Dry the precipitate first in air and then in oven at 110 °C, weigh and report the yield of solid mercury tetrathiocyanato cobaltate (II) complex.

REACTIONS INVOLVED:

Following reactions are involved in the preparation of mercury tetrathiocyanato cobaltate (III) complex.

$$Co(NO_3)_{2.6}H_2O + 4NH_4SCN + HgCl_2 \longrightarrow Hg[Co(SCN)_4] + 2NH_4Cl + 2NH_4OH + 6H_2O$$

RESULTS:

  (i) Physical state: Solid
  (ii) Colour: Blue
  (iii) Yield: ……… g

STRUCTURE:

The structure of mercury tetrathiocyanato cobaltate (II) complex has tetrahedral geometry in which cobalt has sp^3 hybridization. The coordination number of Co in this complex is 4. The thiocyanate ligand is coordinated with Co through S atom. The complex is paramagnetic in nature with 3 unpaired electrons. The blue coloured complex is used for calibrating magnetic balance. The structure of mercury tetrathiocyanato cobaltate (II) complex is shown in figure.
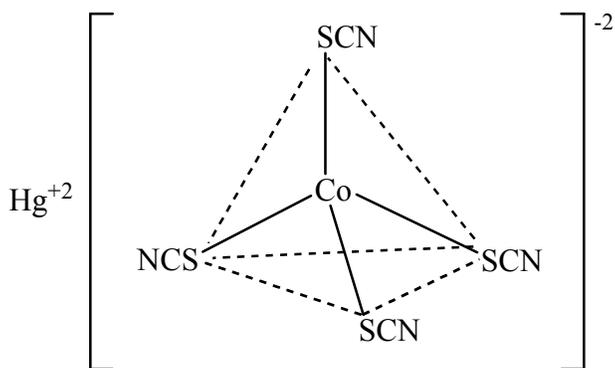
Structure of mercury tetrathiocyanato cobaltate (II) complex

2.9 PREPARATION OF SODIUM DIAMMINE TETRATHIOCYANATO CHROMATE (III) i.e. Na[Cr(NH₃)₂(SCN)₄]

THEORY:

Sodium diammine tetrathiocyanato chromate (III) is a coordination compound having molecular formula $Na[Cr(NH_3)_2(SCN)_4]$. It is obtained by the reaction of Reinecke's salt with sodium perchlorate solution. Therefore, prior to the preparation of the Sodium diamine tetrathiocyanato (III) complex, it is necessary to prepare the Reinecke's salt. Reinecke's salt is a chemical compound with molecular formula $NH_4[Cr(NCS)_4(NH_3)_2] \cdot H_2O$. It is a dark red coloured crystalline compound. The dark-red crystalline compound is soluble in boiling water, acetone, and ethanol. The chromium atom is surrounded by six nitrogen atoms in an octahedral geometry. The NH₃ ligands are mutually *trans* and the Cr–NCS groups are linear. The salt crystallizes with

one molecule of water. It was first prepared in 1863 by treatment of molten NH_4SCN (melting point around 145–150 °C) with $(NH_4)_2Cr_2O_7$.

GLASSWARE AND CHEMICALS REQUIRED:

Glassware and apparatus: Beakers, Buchner funnel, filter flask, conical flask, filter paper, glass rod, vacuum desiccator, refrigerator, metallic spatula.

Reagents and Chemicals: Ammonium thiocyanate (NH_4SCN), Ammonium dichromate (($NH_4)_2Cr_2O_7$), Sodium perchlorate, ice.

PROCEDURE:

Take 8.0 g finely powdered ammonium thiocyanate in a big silica dish heat it until it begins to melt. To this add 3.0 g of finely divided/powdered ammonium dichromate in it in very small quantities. The second proportion of ammonium dichromate should be added only when the previously added proportions reacted completely. The reaction between ammonium thiocyanate and ammonium dichromate is highly exothermic therefore, during the addition of ammonium dichromate care should be taken that the temperature of the reaction should not rise above 160 °C. When addition is completed, cool the silica dish at laboratory temperature and then place it in vacuum desiccator for some time. Take the silica dish out from the vacuum desiccator and break the solid mass with metallic spatula and transfer it in a 100 mL beaker containing 10-15 gm of crushed ice. Stir the mixture vigorously, red coloured solid is formed. Filter out the red coloured solid with Buchner funnel. Recrystallize the solid from hot water, dry and weigh to report the yield. This red coloured solid is Reinecke's salt.

Take 3.0 g of the above prepared Reinecke's salt in a beaker containing 20 mL of hot water to this add 5 mL of sodium perchlorate solution (1 g in 5 mL water). Stir the solution and then cool it in a refrigerator. Filter the precipitate of ammonium perchlorate with Buchner funnel and preserve the aqueous solution of sodium diammine tetrathiocyanato chromate (III).

REACTIONS INVOLVED:

Following reactions are involved in the preparation of Reinecke's salt and sodium diammine tetrathiocyanato chromate (III) complex.

$$NH_4SCN + (NH_4)_2Cr_2O_7 + H_2O \longrightarrow NH_4[Cr(NH_3)_2(SCN)_4].H_2O$$
$$\text{Reinecke's salt}$$

$$NH_4[Cr(NH_3)_2(SCN)_4].H_2O + NaClO_4 \longrightarrow Na[Cr(NH_3)_2(SCN)_4].H_2O + NH_4ClO_4$$
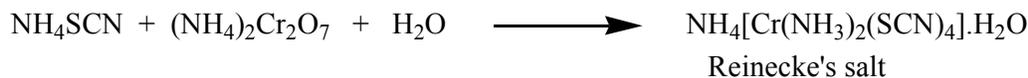
RESULTS:

   (i) Physical state: Solid

   (ii) Colour: ………

   (iii) Yield: ……… g

STRUCTURE:

     Both Reinecke's salt and the sodium diammine tetrathiocyanato chromate (III) complex, Cr (III) are octahedrally surrounded by 6 ligands [4(SCN$^-$) and 2 NH$_3$] with d^{2}sp^3 hybridization. Reinecke's salt and sodium diammine tetrathiocyanato chromate (III) complex are paramagnetic in nature with 3 unpaired electrons. Both will show *cis-* and *trans-* isomerism.

cis-sodium diammine tetrathiocyanato chromate (III)

trans-sodium diammine tetrathiocyanato chromate (III)
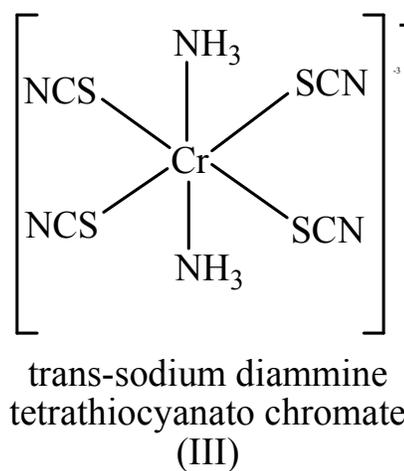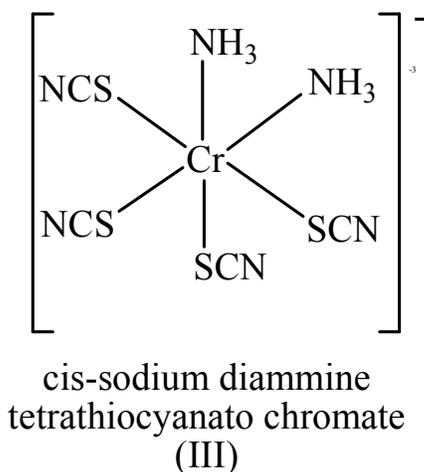
Structure of isomers of sodium diammine tetrathiocyanato chromate (III) complex

2.10   PREPARATION   OF   TRIS   (ACETYLACETONATO) MANGANESE (III) i.e. [Mn(acac)₃]

THEORY:

The Acetylacetonate (acac) is one of the most famous ligands in coordination chemistry. The first appearance of this ligand was in 1890s by Combes. This ligand forms complexes with different coordination and structures with any metal. It is a bidentate ligand. Typically both oxygen atoms bind to the metal to form a six-membered chelate ring.

(keto form)   (enol form)   Formation of chelate

acetylacetone
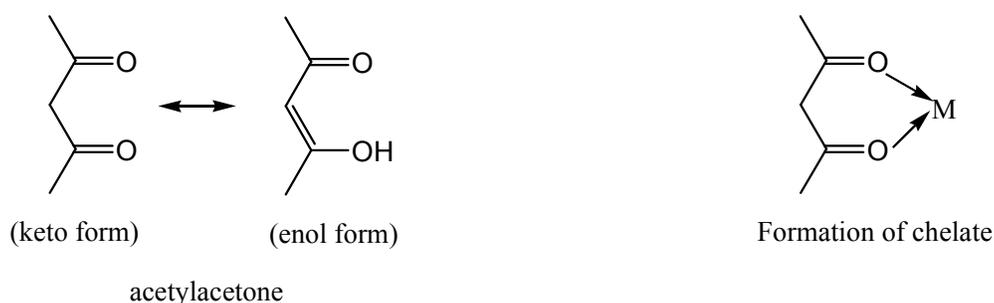
The coordination chemistry of this ligand continues to be studied as the field of materials chemistry grows; these compounds are excellent precursors for metal. Also these complexes are widely used as catalyst precursors and reagents. Cu(I) acac is employed to catalyze Michael additions. The acac/Cu(II) complex is catalyzed coupling and carbene transfer reactions. Different acac complexes were prepared, examples are M(acac)₃ where M= Mn(III), Cr(III), Co(III) and Fe(III) with coordination number 6 and octahedral geometry in which the ligand is usually bonded to the metal through both oxygen atoms.

 Tris(acetylacetonato) manganese (III) complex of Mn with molecular formula [Mn(acac)₃]. It is a shiny dark brown black coloured solid. Its preparation is of two step process. It is prepared by the reaction of Manganese chloride with acetylacetone, sodium acetate and $KMnO_4$.

GLASSWARE AND CHEMICALS REQUIRED:

Glassware and apparatus: Beakers, Buchner funnel, filter flask, conical flask, filter paper, glass rod, ice bath.

Reagents and Chemicals: Manganese chloride tetrahydrate ($MnCl_2.4H_2O$), Sodium acetate (CH_3COONa), Acetylacetone ($CH_3COCH_2COCH_3$), Potassium permanganate ($KMnO_4$), acetone.

PROCEDURE:

To a 250 mL beaker take 2.0 g of manganese chloride tetrahydrate and 5.0 g of sodium acetate, dissolve it in 100 mL of water. To this add 10 mL of acetylacetone under constant stirring. A two phase solution indicates the formation of $Mn(acc)_2$; to this two phase solution add 25 mL of $KMnO_4$ solution (0.5 g of $KMnO_4$ in 25 mL of water). After 10 minutes, add 25 mL of sodium acetate solution (5 g sodium acetate in 25 mL of water) to it in small portions under constant stirring. Heat the solution on water bath for 10-15 minutes (temperature should not increase above 60 °C). Cool the solution on ice bath. Precipitate of shiny brown black crystals of Tris(acetylacetonato) manganese (III) complex is formed. Filter the precipitate with Buchner funnel. Wash it with water and then acetone. Dry, weigh and report the yield of $[Mn(acac)_3]$ complex.

REACTIONS INVOLVED:

The synthesis of Tris(acetylacetonato) manganese (III) complex is a two step process. Following are the reactions involved in the preparation of $[Mn(acac)_3]$ complex.

$$MnCl_2 + 2(CH_3CO)_2CH_2 + 2CH_3COONa \longrightarrow Mn(acac)_2 + 2NaCl + 2CH_3COOH$$

$$Mn(acac)_2 + KMnO_4 + 7(CH_3CO)_2CH_2 + 5CH_3COOH \longrightarrow 5Mn(acac)_3 + CH_3COOK + 4H_2O$$
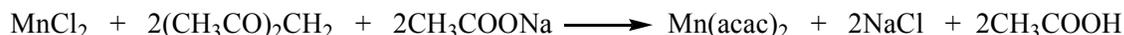
RESULTS:

  (i) Physical state: Solid

  (ii) Colour: shiny brown black crystals

  (iii) Yield: ……… g

STRUCTURE:

Tris(acetylacetonato) manganese (III) complex has 6 coordination number. The central metal Mn(III) is bonded with three acac⁻ (bidentate) ligands. It is paramagnetic in nature with 4 unpaired electrons. It is an outer orbital complex with sp^3d^2 hybridization.

Structure of Tris(acetylacetonato) manganese (III) complex
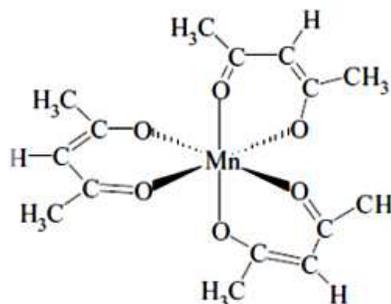
2.11 PREPARATION OF DICHLORO PYRIDINECOBALT (II) i.e. [Co(py)₂Cl₂]

THEORY:

The dichloropyridine cobalt (II) complex is a complex of Co with molecular formula [Co(py)₂Cl₂]. It is prepared by the reaction of anhydrous cobalt chloride with pyridine in alcoholic medium.

GLASSWARE AND CHEMICALS REQUIRED:

**Glassware and apparatus**: Beakers, Buchner funnel, filter flask, conical flask, filter paper, glass rod, vacuum desiccator, vacuum pump.

Reagents and Chemicals: Anhydrous cobalt chloride (CoCl₂), Pyridine (C₅H₅N), Absolute alcohol (ethanol).

PROCEDURE:

Take 4.0 g of anhydrous cobalt chloride in a 100 mL beaker. Dissolve it by adding 20 mL of hot absolute alcohol. In another beaker dissolve 4 mL of pyridine in 12 mL of absolute alcohol. Add the above prepared pyridine solution slowly to the hot alcoholic solution of cobalt chloride. Stir and cool the resulting mixture at laboratory temperature for 30-45 minutes. Formation of precipitate confirms the completion of the reaction. Filter the precipitate with Buchner funnel.

Wash this precipitate with cold ethanol for 3-4 times. Dry the washed precipitate in vacuum desiccator. Weigh and report the yield of dichloropyridine cobalt (II) complex.

REACTIONS INVOLVED:

Preparation of dichloropyridine cobalt (II) complex is a single step process. Following reaction is involved in the preparation of $[Co(py)_2Cl_2]$ complex.

$$CoCl_2 \ + \ 2 \ \underset{N}{\bigcirc} \ \xrightarrow{\text{Ethanol}} \ [Co(py)_2Cl_2]$$
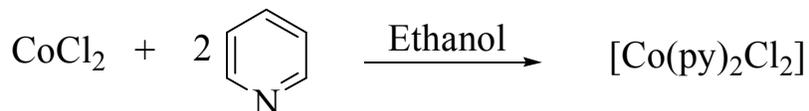
RESULTS:

  (i) Physical state: Solid
  (ii) Colour: ……
  (iii) Yield: ……… g

STRUCTURE:

The dichloropyridine cobalt (II) complex *i.e.* $[Co(py)_2Cl_2]$ has 4 coordination number. The central metal Co(II) is bonded with two py (pyridine) and 2 chloride ligands. It is paramagnetic in nature with 3 unpaired electrons. It is an outer orbital complex with tetrahedral geometry and sp^3 hybridization.
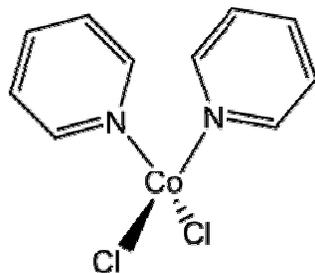
Structure of dichloropyridine cobalt (II) complex *i.e.* $[Co(py)_2Cl_2]$

2.12 PREPARATION OF POTASSIUM TRIOXALATO FERRATE (III) i.e. K$_3$[Fe(C$_2$O$_4$)$_3$]

THEORY:

Potassium ferrioxalate, also known as potassium trisoxalatoferrate(III), is a chemical compound with the formula K$_3$[Fe(C$_2$O$_4$)$_3$], where iron is in the + 3 oxidation state. It is an octahedral transition metal complex in which three bidentate oxalate ions are bonded to an iron center. Potassium acts as a counter ion, balancing the −3 charge of the complex. Crystals of the trihydrated form of the complex, K$_3$[Fe(C$_2$O$_4$)$_3$]·3H$_2$O, are emerald green in colour. In solution, the salt dissociates to give the ferrioxalate anion, [Fe(C$_2$O$_4$)$_3$]$^{-3}$, which appears fluorescent green in colour. Potassium ferrioxalate is often used in the measurement of light flux. The complex can be synthesized by the reaction between ferrous ammonium sulphate, oxalic acid and potassium oxalate.

GLASSWARE AND CHEMICALS REQUIRED:

Glassware and apparatus: Beakers, Buchner funnel, filter flask, conical flask, filter paper, glass rod, water bath.

Reagents and Chemicals: Ferrous ammonium sulphate (FeSO$_4$.(NH$_4$)$_2$SO$_4$), Oxalic acid (HOOC-COOH), Potassium oxalate (KOOC-COOK), Hydrogen peroxide (H$_2$O$_2$), dil. H$_2$SO$_4$, Ethanol, Distilled water.

PROCEDURE:

In a 125-mL conical flask, 5.0 g of ferrous ammonium sulfate hexahydrate is dissolved in 20 mL distilled water. The solution is acidified by the addition of several drops of dilute sulphuric acid. To this solution, add 30 mL of oxalic acid solution (3.0 g oxalic acid in 30 mL of distilled water) and heat to boiling while stirring constantly to prevent bumping. Remove from heat and allow the ferrous oxalate to settle and decant the solid product from the liquid. Wash the precipitate twice with 20 mL hot distilled water. Add 15 mL of saturate potassium oxalate solution at room temperatuer to the flask containing the precipitate of ferrous oxaltae, stir and heat to 40°C. At

this temperature (*i.e.* 40°C), add 20 mL of 3% hydrogen peroxide dropwise to this solution under constant stirring. Add another portion of 20 mL of 3% hydrogen peroxide dropwise to this solution. After addition is complete, heat the resulting solution to boiling. Prepare 10 mL of 1M oxalic acid solution (1.26 g oxalic acid in 10 mL of distilled water); take out 5 mL of 1M oxalic solution from that and add all 5 mL at once to the boiling solution. Stir continuously and then continue to add the 5 mL of 1M oxalic solution dropwise while boiling. The colour of the solution turns to green. Filter the hot green coloured solution into a conical flask. To this add about 20 mL of ethanol and heat the mixture about 70 °C. Add additional amount of ethyl alcohol continuously under constant stirring until the solution turns cloudy. Allow the solution to cool in a dark place to get crystallize. Filter the resulting crystals with Buchner funnel. Wash the crystalline material with 1:1 mixture ethanol water. Dry the crystals on filter paper, weigh and report the yield.

REACTIONS INVOLVED:

The synthesis of potassium trioxalato ferrate (III) is achieved in two steps. The first step involves the preparation of yellow iron (II) oxalate from the reaction of ferrous ammonium sulphate and oxalic acid in the presence of dil. H_2SO_4. The second step involves the oxidation of ferrous oxalate with H_2O_2 in the presence of excess of oxalate ions to produce green crystals of potassium trioxalato ferrate (III). The reactions involved are shown as follow:

RESULTS:

(i)   Physical state: Solid
(ii) Colour: ……
(iii) Yield: ……… g
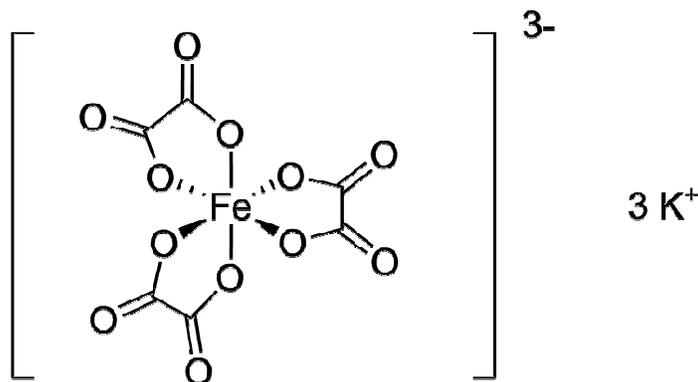
STRUCTURE:

Structure of potassium trioxalato ferrate (III) complex

2.13 PREPARATION OF HEXAAMMINE NICKEL (II) CHLORIDE i.e. [Ni(NH₃)₆]Cl₂

2.13 PREPARATION OF HEXAAMMINE NICKEL (II) CHLORIDE i.e. $[Ni(NH_3)_6]Cl_2$

THEORY:

In aqueous solution, nickel ion Ni^{2+} is surrounded by six water molecules which are actually bonded to the central metal ion. This complex is called the hexaquonickel (II) ion. When aqueous nickel chloride is precipitated from solution, the nickel ions carry their six water molecules into the crystals, and hence solid nickel (II) chloride is hydrated. It has the formula $[Ni(H_2O)_6]Cl_2$, and is more properly called hexaquonickel (II) chloride. When ammonia is added to a solution of this salt, the ammonia molecules compete with the water in bonding Ni^{2+}, and because the ammonia forms a stronger bond than water, the ammonia replaces the water molecules to form hexaammine Nickel (II) chloride complex :

GLASSWARE AND CHEMICALS REQUIRED:

Glassware and apparatus: Beakers, Buchner funnel, filter flask, conical flask, filter paper, glass rod, ice bath.

Reagents and Chemicals: Nickel chloride hexahydrate ($NiCl_2.6H_2O$), Ammonia solution (NH_4OH), Ethanol, Distilled water, ice.

PROCEDURE:

In a 100 mL beaker, take 6.0 g of nickel chloride hexahydrate and dissolve it in 20 mL of distilled water. Stir this solution. Add 15 ml of concentrated aqueous ammonia, slowly, to a rapidly stirred solution of nickel chloride. Make sure that the colour of the solution has changed from pale green to intense violet (if not, add more ammonia). Allow the solution to stand at room temperature for 5 minutes. Cool in an ice-bath, without disturbance, for about 15-20 minutes. Add about 50 mL of ethanol in it to complete the deposition. Filter the crystals with Buchner funnel, and wash the crystals with ethanol. Dry the crystals by placing them between pieces of filter paper. Determine the weight. Report the yield.

REACTIONS INVOLVED:

Following reaction is involved in the preparation of hexaammine nickel(II) chloride comolex.

$$NiCl_2.H_2O \ + \ 6NH_3 \longrightarrow [Ni(NH_3)_6]Cl_2 \ + \ 6H_2O$$
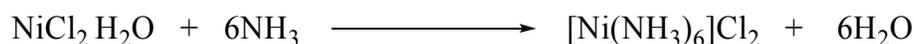
RESULTS:

(i) Physical state: Solid
(ii) Colour: Voilet
(iii) Yield: ……… g

STRUCTURE:

In hexaammine nickel(II) chloride comolex Ni is bonded to 6 ammonia molecues. The coordination number of nickel is 6, hence it has octahedral geometry with sp^3d^2 hybridization. It is a paramagnetic complex with 2 unpaired electrons.
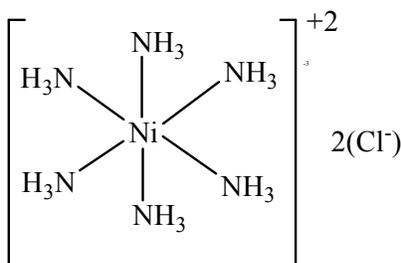
Structure of hexaammine nickel(II) chloride comolex

2.14 SUMMARY

- In the present unit students would have learnt the laboratory preparation methods of various inorganic compounds specially the coordination complex.

- Students also learnt how to write the laboratory reports in a proper sequential manner.

- Present chapter also described the structure and hybridization of the various coordination complexes.

- The magnetic properties of the prepared complex have also mentioned at the end of each preparation that will certainly help improve their understanding towards the subject.

- At the end of each preparation structure of the prepared complex is also presented which helps to understand how the ligands are distributed in space around the central metal ion.

UNIT 3: QUANTITATIVE ESTIMATIONS

CONTENTS:

3.1 OBJECTIVES

In this unit learner will be able to

❖ Learn about the complexometric titration

❖ Prepare and standardize the EDTA solution

❖ Know the importance of indicators in complexometric titration

❖ Select the suitable masking and demasking agents for complexometric titration

❖ Learn how to perform the estimation of the various metal ions

3.2 INTRODUCTION

3.2.1 COMPLEXOMETRIC TITRATION

The technique involves titrating metal ions with a complexing agent or chelating agent (Ligand) and is commonly referred to as complexometric titration. This method represents the analytical application of a complexation reaction. In this method, a simple ion is transformed into a complex ion and the equivalence point is determined by using metal indicators or electrometrically. Various other names such as **chilometric titrations**, chilometry, chilatometric titrations and EDTA titrations have been used to describe this method. All these terms refer to same analytical method and they have resulted from the use of EDTA (Ethylene diamine tetra acetic acid) and other chilons. These chilons react with metal ions to form a special type of complex known as **chelate**. Complexometric titration is a form of volumetric analysis in which the formation of a colored complex is used to indicate the end point of a titration. Complexometric titrations are particularly useful for the determination of a mixture of different metal ions in solution. An indicator capable of producing an unambiguous colour change is usually used to detect the end-point of the titration.

3.2.2 REACTIONS FOR COMPLEXOMETRIC TITRATION

In theory, any complexation reaction can be used as a volumetric technique provided that:
- The reaction reaches equilibrium rapidly after each portion of titrant is added.
- The interfering situations do not arise. For instance, the stepwise formation of several different complexes of the metal ion with the titrant, resulting in the presence of more than one complex in solution during the titration process.
- A complexometric indicator capable of locating equivalence point with fair accuracy is available.

In practice, the use of EDTA as a titrant is well established

3.2.3 COMPLEX TITRATION WITH EDTA

EDTA, ethylenediaminetetraacetic acid, has four carboxyl groups and two amine groups that can act as electron pair donors, or Lewis bases. The ability of EDTA to potentially donate its six lone pairs of electrons for the formation of coordinate covalent bonds to metal cations makes EDTA a

hexadentate ligand. However, in practice EDTA is usually only partially ionized, and thus forms fewer than six coordinate covalent bonds with metal cations. Disodium EDTA is commonly used to standardize aqueous solutions of transition metal cations. Disodium EDTA (often written as **Na₂H₂Y**) only forms four coordinate covalent bonds to metal cations at pH values ≤ 12. In this pH range, the amine groups remain protonated and thus unable to donate electrons to the formation of coordinate covalent bonds.
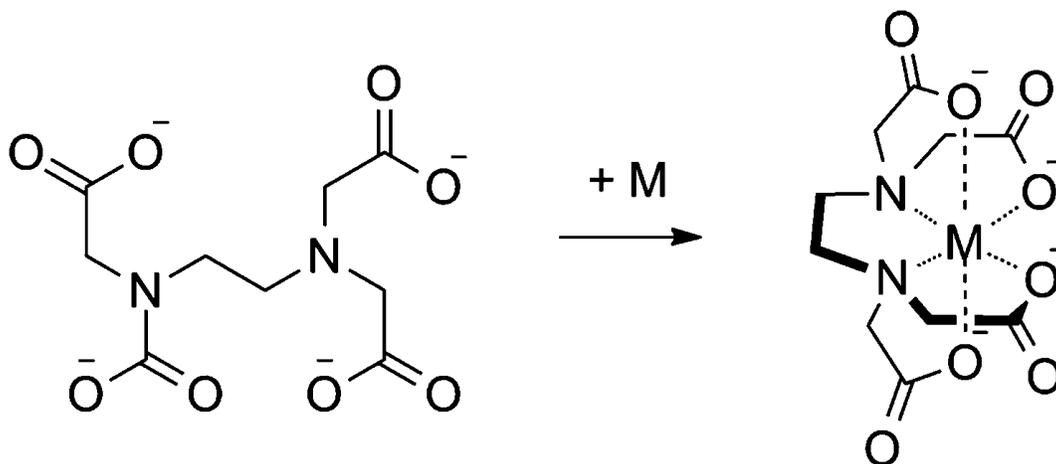
3.2.4 TYPES OF COMPLEXOMETRIC TITRATIONS

Complexometric titrations are of 4 types:

1. DIRECT TITRATION

It is the simplest and the most convenient method used in chelometry. In this method, the standard chelon solution is added to the metal ion solution until the end point is detected. This method is analogous to simple acid-base titrations. E.g.-calcium gluconate injection, calcium lactate tablets and compound sodiumlactate injection for the assay of calcium chloride ($CaCl_2.6H_2O$).

Limitations

- slow complexation reaction
- Interference due to presence of other ions

2. BACK TITRATION

In this method, excess of a standard EDTA solution is added to the metal solution, which is to be analyzed, and the excess is back titrated with a standard solution of a second metal ion. e.g.- Determination of Mn. This metal cannot be directly titrated with EDTA because of precipitation of $Mn(OH)_2$. An excess of known volume of EDTA is added to an acidic solution of Mn salt and then ammonia buffer is used to adjust the pH to 10 and the excess EDTA remaining after chelation, is back titrated with a standard Zn solution kept in burette using Eriochrome black-T as indicator. This method is analogous to back titration method in acidimetry. e.g.- ZnO

3. REPLACEMENT TITRATION

In this method the metal, which is to be analyzed, displaces quantitatively the metal from the complex. When direct or back titrations do not give sharp end points, the metal may be determined by the displacement of an equivalent amount of Mg or Zn from a less stable EDTA complex.

$$Mn^{+2} \quad + \quad Mg\ EDTA^{-2} \quad \longrightarrow \quad Mg^{+2} \quad + \quad Mn\ EDTA^{-2}$$
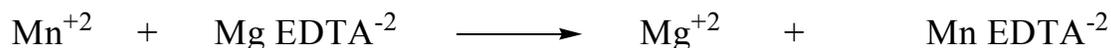
Mn displaces Mg from Mn EDTA solution. The freed Mg metal is then directly titrated with a standard EDTA solution. In this method, excess quantity of Mg EDTA chelate is added to Mn solution. Mn quantitatively displaces Mg from Mg EDTA chelate. This displacement takes place because Mn forms a more stable complex with EDTA. By this method Ca, Pb, Hg may be determined using Eriochrome black-T indicator.

4. INDIRECT TITRATION

This is also known as Alkalimetric titration. It is used for the determination of ions such as anions, which do not react with EDTA chelate. Protons from disodium EDTA are displaced by a heavy metal and titrated with sodium alkali.

$$Mn^{+} \quad + H_2X^{-2} \quad \rightarrow \quad MX^{(n-4)} \quad + \quad 2H^{+}$$

e.g. - Barbiturates do not react with EDTA but are quantitatively precipitated from alkaline solution by mercuric ions as 1:1 complex.

3.2.5 PURIFICATION OF DISODIUM EDTA

Commercial samples of disodium EDTA may be purified for use as a primary standard by adding ethanol to a saturated aqueous solution until the first permanent precipitate appears; filter and add an equal volume of ethanol; filter the precipitated disodium EDTA, wash with acetone and ether, and dry to constant weight at 80°C, drying may require four days. The official material contains not less than 98% of the dihydrate.

3.2.6 PREPARATION OF M/20 DISODIUM EDTA

Dissolve 18.6 g of disodium EDTA in water and make the volume upto 1000 mL and standardize the prepared solution.

3.2.7 STANDARDIZATION OF DISODIUM EDTA

Weigh accurately about 0.2 g of $CaCO_3$ in a titration flask. Add 50 mL of water and minimum quantity of dil. HCl to dissolve $CaCO_3$. Adjust the pH of the solution to 12 by adding NaOH. Add 0.3 g of hydroxyl naphthol blue indicator and titrate with the prepared M/20 disodium EDTA solution, until the solution is deep blue in colour. The HCl solubilizes the $CaCO_3$ by converting it to $CaCl_2$. The NaOH makes the solution alkaline and maintains the pH at about 12 so that the Ca-EDTA complex would be stable and any Mg, which might be present as a contaminant, would not react. The coloured Ca-indicator complex gives up Ca to EDTA, liberating the free uncomplexed indicator, which is blue.

3.2.8 FACTORS INFLUENCING EDTA REACTIONS

- The nature and activity of metal ion.
- The pH at which the titration is carried out.
- The presence of interfering ions such as CN^-, Citrate, Tartrate, F^- and other complex forming agents.
- Organic solvents also increase the stability of complex.

Note that the shorthand form **Na$_{4-x}$H$_x$Y** can be used to represent any species of EDTA, with **x** designating the number of acidic protons bonded to the EDTA molecule. EDTA forms an octahedral complex with most 2^+ metal cations, M^{2+}, in aqueous solution. The main reason that

EDTA is used so extensively in the standardization of metal cation solutions is that the formation constant for most metal cation-EDTA complexes is very high, meaning that the equilibrium for the reaction:

$$M^{2+} + H_4Y \rightarrow MH_2Y + 2H^+$$
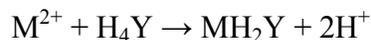
The reaction lies always towards the forward direction. Carrying out the reaction in a basic buffer solution removes H^+ as it is formed, which also favours the formation of the EDTA-metal cation complex reaction product. For most purposes it can be considered that the formation of the metal cation-EDTA complex goes to completion, and this is chiefly why EDTA is used in titrations /standardizations of this type.

3.3 METAL ION INDICATORS

To carry out metal cation titrations using EDTA, it is almost always necessary to use a complexometric indicator to determine when the end point has been reached. Common indicators are organic dyes such as Fast Sulphon Black, Eriochrome Black T. Colour change shows that the indicator has been displaced (usually by EDTA) from the metal cations in solution when the endpoint has been reached. Thus, the free indicator (rather than the metal complex) serves as the endpoint indicator. The requisites of a metalion indicator for use in the visual detection of end points include:

- The colour reaction must be before the end point, when nearly all the metal ion is complexed with EDTA, the solution is strongly coloured.
- The colour reaction should be specific or selective.
- The metal-indicator complex must possess sufficient stability, otherwise, due to dissociation; a sharp colour change is not attained. The metal-indicator complex must, however, be less stable than the metal-EDTA complex to ensure that, at the end point, EDTA removes metal ions from the metal indicator-complex. The change in equilibrium from the metal indicator complex to the metal-EDTA complex should be sharp and rapid.
- The colour contrast between the free indicator and the metal-indicator complex should be readily observed.
- The indicator must be very sensitive to metal ions (i.e. to pM) so that the colour change occurs as near to equivalence point as possible.

- The above requirements must be fulfilled within the pH range at which the titration is performed.

3.4 TITRATION OF MIXTURES, SELECTIVITY, MASKING AND DEMASKING AGENTS

EDTA is a very unselective reagent because it complexes with numerous doubly, triply and quadruply charged cations. When a solution containing two cations which complex with EDTA is titrated without the addition of a complex-forming indicator, and if a titration error of 0.1 per cent is permissible, then the ratio of the stability constants of the EDTA complexes of the two metals M and N must be such that KM/KN > 106 if N is not to interfere with the titration of M. Strictly, of course, the constants KM and KN considered in the above expression should be the apparent stability constants of the complexes. If complex-forming indicators are used, then for a similar titration error KM/KN > 108.The following procedures will help to increase the selectivity:

(A) SUITABLE CONTROL OF THE pH OF THE SOLUTION

This, of course, makes use of the different stabilities of metal-EDTA complexes. Thus bismuth and thorium can be titrated in an acidic solution (pH = 2) with xylenol orange or methyl thymol blue as indicator and most divalent cations do not interfere. A mixture of bismuth and lead ions can be successfully titrated by first titrating the bismuth at pH 2 with xylenol orange as indicator, and then adding hexamine to raise the pH to about 5, and titrating the lead.

(B) USE OF MASKING AGENTS

Masking may be defined as the process in which a substance, without physical separation of it or its reaction products, is so transformed that it does not enter into a particular reaction. Demasking is the process in which the masked substance regains its ability to enter into a particular reaction. By the use of masking agents, some of the cations in a mixture can often be 'masked' so that they can no longer react with EDTA or with the indicator. An effective masking agent is the cyanide ion; this forms stable cyanide complexes with the cations of Cd, Zn, Hg(II), Cu, Co, Ni, Ag, and the platinum metals, but not with the alkaline earths, manganese, and lead

It is therefore possible to determine cations such as Ca^{2+}, Mg^{2+}, Pb^{2+}, and Mn^{2+} in the presence of the above-mentioned metals by masking with an excess of potassium or sodium cyanide. A small amount of iron may be masked by cyanide if it is first reduced to the iron(II) state by the addition of ascorbic acid. Titanium(IV), iron(III), and aluminium can be masked with triethanolamine; mercury with iodide ions; and aluminium, iron(III), titanium(IV), and tin(II) with ammonium fluoride (the cations of the alkaline-earth metals yield slightly soluble fluorides). Sometimes the metal may be transformed into a different oxidation state: thus copper(II) may be reduced in acid solution by hydroxylamine or ascorbic acid. After rendering ammoniacal, nickel or cobalt can be titrated using, for example, murexide as indicator without interference from the copper, which is now present as Cu(I). Iron(III) can often be similarly masked by reduction with ascorbic acid.

(C) SELECTIVE DEMASKING

The cyanide complexes of zinc and cadmium may be demasked with formaldehyde, acetic acid solution or, better, with chloral hydrate: The use of masking and selective demasking agents permits the successive titration of many metals. Thus a solution containing Mg, Zn, and Cu can be titrated as follows:

- Add excess of standard EDTA and back-titrate with standard Mg solution using solochrome black as indicator. This gives the sum of all the metals present.

- Treat an aliquot portion with excess of KCN (Poison!) and titrate as before. This gives Mg only.

- Add excess of chloral hydrate (or of formaldehyde-acetic acid solution) to the titrated solution in order to liberate the Zn from the cyanide complex, and titrate until the indicator turns blue. This gives the Zn only. The Cu content may then be found by difference.

3.5 EFFECT OF pH ON COMPLEX FORMATION

Ethylenediamine tetra-acetic acid ionizes in four stages ($pK_1=2.0$, $pK_2=2.67$, $pK_3=6.16$ and $pK_4=10.26$) and, since the actual complexing species is Y^{4-}, complexes will form more efficiently and be more stable in alkaline solution. If, however, the solubility product of the metal hydroxide is low, it may be precipitated if the hydroxyl ion concentration is increased too much. On the other hand, at lower pH values when the concentration of Y^{4-} is lower, the stability

constant of the complexes will not be so high. Complexes of most divalent metals are stable in ammonical solution. Those of the alkaline earth metals, such as copper, lead and nickel, are stable down to pH 3 and hence can be titrated selectively in the presence of alkaline earth metals. Trivalent metal complexes are usually still more firmly bound and stable in strongly acid solutions; for example, the cobalt(III) complex of EDTA is stable in concentrated hydrochloric acid. Although most complexes are stable over a fair range of pH, solutions are usually buffered 6 at a pH at which the complex is stable and at which the colour change of the indicator is most distinct.

3.6 PRINCIPLE OF COMPLEXOMETRIC TITRATION

Many principles of acid-base titrations are used in complexometric titration. In complexometric titration, the free metal ions disappear as they are changed into complex ions. In acid-base titrations, the end point is marked by sudden change in pH. Similarly, in EDTA titration, if we plot pM (negative log of metal ion concentration) v/s volume of titrant, we will find that at the end point, the pM rapidly increases (Fig. 1). This sudden pM raise results from removal of traces of metal ions from solution by EDTA. Any method, which can determine this disappearance of free metal ions, can be used to detect end point in complexometric titrations. End point can be detected usually with an indicator or instrumentally by potentiometric or conductometric (electrometric) method.
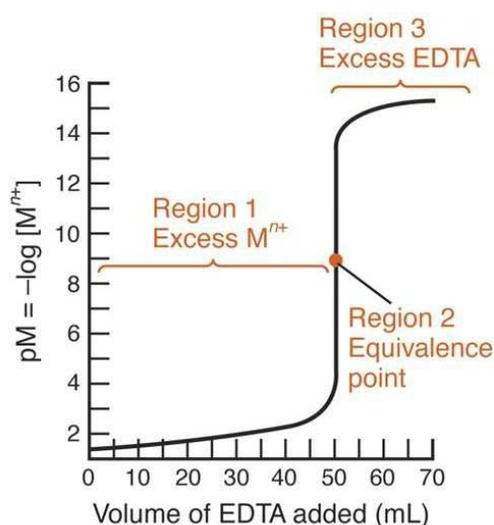
Fig. 1: Titration curve of EDTA titration

3.6.1 METHODS OF END POINT DETECTION

End point in complexometric titration can be detected by the following two methods:

The end point in complexometric titrations is shown by means of pM indicators. The concept of pM arises as follows:

If K is the stability constant,

$$K = [MX]/[M][X]$$

then, $[M] = [MX]/[X]K$

or $\log [M] = \log [MX]/[X] - \log K$

and $pM = \log [X]/[MX] - pK$

Therefore, if a solution is made such that [X] = [MX], pM = -pK (or pM = pK', where K' = dissociation constant). This means that, in a solution containing equal activities of metal omplex and free chelating agent, the concentration of metal ions will remain roughly constant and will be buffered in the same way as hydrogen ions in a pH buffer. Since, however, chelating agents are also bases; equilibrium in a metal-buffer solution is often greatly affected by a change in pH. In general, for chelating agents of the amino acid type (e.g., edetic acid and ammonia triacetic acid), it may be said that when [X] = [MX], pM increases with pH until about pH 10, when it attains a constant value. This pH is, therefore, usually chosen for carrying out titrations of metals with chelating agents in buffered solutions. The pM indicator is a dye which is capable of acting as a chelating agent to give a dye-metal complex. The latter is different in colour from the dye itself and also has a low stability constant than the chelate-metal complex. The colour of the solution, therefore, remains that of the dye complex until the end point, when an equivalent amount of sodium EDTA has been added. As soon as there is the slightest excess of EDTA, the metal-dye complex decomposes to produce free dye; this is accomplished by a change in colour.

3.6.2 MECHANISM OF ACTION OF INDICATOR

Let the metal be denoted by M, indicator by I and chelate by EDTA. At the onset of the titration, the reaction medium contains the metal-indicator complex (MI) and excess of metal ion. When EDTA titrant is added to the system, a competitive reaction takes place between the free metal ions and EDTA. Since the metal-indicator complex (MI) is weaker than the metal-EDTA chelate, the EDTA which is being added during the course of the titration is chelating the free metal ions

in solution at the expense of the MI complex. Finally, at the end point, EDTA removes the last traces of the metal from the indicator and the indicator changes from its complexed colour to its metal free colour. The overall reaction is given by:

MI   +   EDTA $\longrightarrow$ M-EDTA   +   I
(Metal-indicator complex)   (Free indicator)
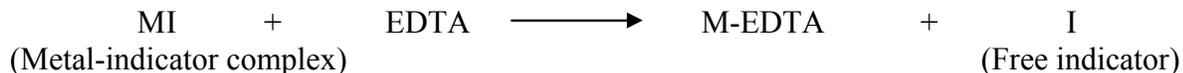
3.7 ESTIMATION OF Ca-Mg IN THE GIVEN MIXTURE SOLUTION OF BOTH BY TITRATION WITH EDTA

THEORY

Many metal ions react with electron pair donors to form coordination compounds or complex ions. The formation of a particular class of coordination compounds, called chelates, are especially well suited for quantitative methods. A chelate is formed when a metal ion coordinates with two (or more) donor groups of a single ligand. Tertiary amine compounds such as ethylenadiaminetetraacetic acid (EDTA) are widely used for the formation of chelates. Complexometric titrations with EDTA have been reported for the analysis of nearly all metal ions. Because EDTA has four acidic protons, the formation of metal-ion/EDTA complexes is dependent upon the pH. For the estimation of Ca^{2+} - Mg^{2+}, one must buffer the solution to a pH of 10 so that complex formation will be quantitative. The reaction of M^{2+} (M = Ca and Mg) with EDTA may be expressed as:

$$M^{2+}   +   H_2Y^{2-} \longrightarrow MY^{-2}   +   2H^+$$
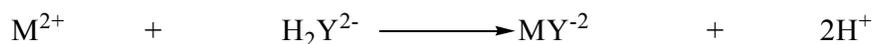
The endpoint of the titration is determined by the addition of Eriochrome Black T, which forms a colored chelate with Mg^{2+} and undergoes a color change when the Mg^{2+} is released to form a chelate with EDTA. However, murexide indicator is used to estimate the Ca^{+2} ions which forms a colored chelate with Ca^{2+} and undergoes a colour change when the Ca^{2+} is released to form a chelate with EDTA. While it is possible to achieve relatively good results by titration with EDTA prepared directly from the solid, better results should be obtained when the EDTA is standardized against a solution containing a known amount of metal ion.

REAGENTS AND APPARATUS:

Chemicals and Reagents:

Mixture solution of Ca^{+2} and Mg^{+2} ions; Dil. HCl solution; 0.01 M EDTA solution; Buffer solution of pH 10; NaOH solution (2N); Eriochrome Black-T and murexide indicator, distilled water.

Glassware and Apparatus:

Beakers, glass rod, conical flasks, volumetric flasks, burette, pipette, dropper.

PROCEDURE

Prepare 50 mL mixture solution of Ca^{+2} and Mg^{+2} [25mL Ca^{+2} solution ($CaCO_3$ 10 g/L), and 25 mL Mg^{+2} solution (MgO 4g/L or $MgCO_3$ 8.4 g/L); add minimum quantity of dilute HCl to it] in a beaker. Transfer the solution to a volumetric flask of 250 mL. Make up the solution up to 250 mL. This solution is now termed as **unknown solution**. To prepare the 0.01M buffer solution of pH 10, Take 200 mL beaker, dissolve 7g of NH_4Cl in 56.8 mL of conc. NH_3 solution and dilute it to 100 mL. Fill the standard EDTA solution in Burette.

Pipette out 25 mL of unknown solution in a conical flask. Add 10 mL buffer of pH 10 to it. Dilute the resultant solution about 50 mL by adding 15 mL of distilled water. Now add few drops of Eriochrome Black-T indicator to it and warm the resultant solution about 60°C and titrate it with standard EDTA solution until the colour changes from red to blue. Note the reading of burette. Repeat the above titration procedure up to 3 times, and note down the readings of burette for all runs.

Pipette out another 25 mL of unknown solution in a conical flask. Add 25 mL of distilled water to it. Now add 5 mL of 2N NaOH solution to it and few drops of murexide indicator; titrate the resultant solution with standard EDTA solution until the colour changes from red to violet. Note down the burette readings. Repeat the above titration procedure up to 3 times, and note down the readings of burette for all runs.

OBSERVATION TABLE

Observation Table for EDTA titration using Eriochrome Black-T (For estimation of Mg)

S. No.	Titration runs	Volume of unknown solution (mL)	Volume of EDTA used (mL) (Burette reading) **X**
1	1st	25 mL	
2	2nd	25 mL	
3	3rd	25 mL	

Observation Table for EDTA titration using murexide indicator (for estimation of Ca)

S. No.	Titration runs	Volume of unknown solution (mL)	Volume of EDTA used (mL) (Burette reading) **Y**
1	1st	25 mL	
2	2nd	25 mL	
3	3rd	25 mL	

CALCULATION:

1.   1 mL of 1mole of EDTA ≡ 1 mole of Ca

                        1 mL of 1mole of EDTA ≡ 40.08 g of Ca

                   1 mL of 0.01mole of EDTA ≡ 0.4008 g of Ca

Y mL of 0.01 mole EDTA ≡ **Y × 0.40 g** of Ca present in 25 mL of unknown solution

2.   1 mL of 1mole of EDTA ≡ 1 mole of Mg

                        1 mL of 1mole of EDTA ≡ 24.32 g of Mg

                   1 mL of 0.01mole of EDTA ≡ 0.2432 g of Mg

X-Y mL of 0.01 mole EDTA ≡ **(X-Y) × 0.2432 g** of Mg present in 25 mL of unknown solution

RESULTS:

Amount of Mg present in given unknown solution is …………g/25 mL of H_2O

Amount of Ca present in given unknown solution is …………g/25 mL of H_2O

3.8 ESTIMATION OF Mg IN THE GIVEN MIXTURE SOLUTION OF BOTH BY TITRATION WITH EDTA

REAGENTS AND APPARATUS:

Chemicals and Reagents:

0.01M Solution of Magnesium sulphate; 0.01 M EDTA solution; Buffer solution of pH 10; Eriochrome Black-T; distilled water

Glassware and Apparatus:

Beakers, glass rod, conical flasks, volumetric flasks, burette, pipette, dropper.

PROCEDURE

Prepare 250 mL solution of 0.01 M Magnesium sulphate salt ($MgSO_4.7H_2O$; MW=246.5) dissolve 2.46 g of Magnesium sulphate in 250 mL of distilled water in a 250 mL measuring flask. To prepare 0.01M buffer solution of pH 10, take 200 mL beaker dissolve 7g of NH_4Cl in 56.8 mL of conc. NH_3 solution and dilute it to 100 mL. To prepare 0.01M EDTA solution, dissolve 0.93 g EDTA in 250 mL of distilled water. Take 10 mL of prepared magnesium solution in a conical flask. To this add 10 mL of buffer solution and 4-5 drops of indicator, it gives red coloured solution. Titrate the resultant mixture with EDTA solution from the burette gradually till the colour of the solution completely changes from red to blue. Note the reading of burette and repeat the titration for three runs to achieve the three concordant readings.

OBSERVATION TABLE

Observation Table for EDTA titration using Eriochrome Black-T

S. No.	Titration runs	Volume of unknown solution (mL)	Volume of EDTA used (mL) (Burette reading) **X**
1	1st	10 mL	
2	2nd	10 mL	
3	3rd	10 mL	

CALCULATION:

$$1 \text{ mL of 1mole of EDTA} \equiv 1 \text{ mole of Mg}$$

$$1 \text{ mL of 1mole of EDTA} \equiv 24.32 \text{ g of Mg}$$

$$1 \text{ mL of 0.01mole of EDTA} \equiv 0.2432 \text{ g of Mg}$$

X mL of 0.01 mole EDTA ≡ **X** × **0.2432 g** of Mg present in 10 mL of unknown solution

RESULTS:

Amount of Mg present in given unknown solution is …………g/10 mL of H_2O

Note: Similarly Ca can also be estimated.

3.9   ESTIMATION   OF   Zn-Mg   IN   THE   GIVEN   MIXTURE SOLUTION OF BOTH BY TITRATION WITH EDTA

THEORY:

The estimation of Zn^{+2} and Mg^{+2} ions are based on the complex formation with EDTA. Hence it is also comes under complexometric titration methods of estimation. Disodium salt of EDTA is used for complexometric titration. The structure of a complex formed by a divalent ion with EDTA is shown as:
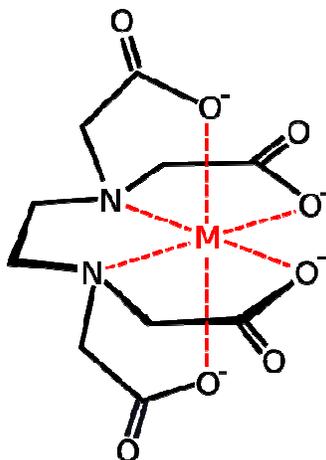
Structure of a divalent metal–EDTA complex

REAGENTS AND APPARATUS:

Chemicals and Reagents:

Mixture solution of Zn^{+2} and Mg^{+2} ions; Dil. 1M HCl solution; 0.01 M EDTA solution; Buffer solution of pH 10; NaOH solution (2N); Eriochrome Black-T indicator, distilled water.

Glassware and Apparatus:

Beakers, glass rod, conical flasks, volumetric flasks, burette, pipette, dropper.

PROCEDURE

Prepare 50 mL mixture solution of Zn^{+2} and Mg^{+2} [25mL Zn^{+2} solution (ZnO 8 g/L), and 25 mL Mg^{+2} solution (MgO 4g/L or $MgCO_3$ 8.4 g/L); add minimum quantity of dilute HCl to it] in a beaker. Transfer the solution to a volumetric flask of 250 mL. Make up the solution up to 250 mL. This solution is now termed as **unknown solution**. To prepare the 0.01M buffer solution of pH 10, Take 200 mL beaker dissolve 7g of NH_4Cl in 56.8 mL of conc. NH_3 solution and dilute it to 100 mL. Fill the standard EDTA solution in Burette.

Pipette out 25 mL of unknown solution in a conical flask. Add 10 mL buffer of pH 10 to it. Dilute the resultant solution about 50 mL by adding 15 mL of distilled water. Now add 4-5 drops of Eriochrome Black-T indicator to it and warm the resultant solution about 60°C and titrate it with standard EDTA solution until the colour changes from red to blue. Note the reading of

burette. Repeat the above titration procedure up to 3 times, and note down the readings of burette for all runs.

Pipette out another 25 mL of unknown solution in a conical flask. Add 25 mL of distilled water to it. Now add 5 mL of 2N NaOH solution to it and few drops of murexide indicator; titrate the resultant solution with standard EDTA solution until the colour changes from red to violet. Note down the burette readings. Repeat the above titration procedure up to 3 times, and note down the readings of burette for all runs.

OBSERVATION TABLE

Observation Table for EDTA titration (without adding NaOH solution) (estimation of Mg)

S. No.	Titration runs	Volume of unknown solution (mL)	Volume of EDTA used (mL) (Burette reading) X
1	1st	25 mL	
2	2nd	25 mL	
3	3rd	25 mL	

Observation Table for EDTA titration (with NaOH solution) (for estimation of Zn)

S. No.	Titration runs	Volume of unknown solution (mL)	Volume of EDTA used (mL) (Burette reading) Y
1	1st	25 mL	
2	2nd	25 mL	
3	3rd	25 mL	

CALCULATION:

1.   1 mL of 1mole of EDTA ≡ 1 mole of Zn

                        1 mL of 1mole of EDTA ≡ 65.38 g of Zn

                1 mL of 0.01mole of EDTA ≡ 0.6538 g of Zn

Y mL of 0.01 mole EDTA ≡ **Y × 0.6538 g** of Zn present in 25 mL of unknown solution

2.   1 mL of 1mole of EDTA ≡ 1 mole of Mg

                        1 mL of 1mole of EDTA ≡ 24.32 g of Mg

1 mL of 0.01mole of EDTA ≡ 0.2432 g of Mg

X-Y mL of 0.01 mole EDTA ≡ **(X-Y) × 0.2432 g** of Mg present in 25 mL of unknown solution

RESULTS:

Amount of Mg present in given unknown solution is …………g/25 mL of H_2O

Amount of Zn present in given unknown solution is …………g/25 mL of H_2O

3.10 SUMMARY

- In the present unit students would have learnt the fundamentals and principle of complexometric titration.

- Students also learnt how the ETDA forms complexes with various metal ions

- Students also learnt how to prepare and standardize the ETDA solution.

- Present chapter also described the importance and proper selection of masking and demasking agents.

- The chapter has also described the method of end point determination in complexometric titration.

- At the end students would have also learnt the estimation of various metal ions by complexometric titration method.

UNIT 4: SEPARATION AND IDENTIFICATION OF COMPONENTS OF BINARY ORGANIC MIXTURES

CONTENTS:

4.1 OBJECTIVES

In this unit learner will be able to

❖ Learn about the need for separation of organic compounds

❖ Know about the types of separation techniques for organic binary mixture

❖ Learn about the systematic separation procedure of binary organic mixture

❖ Learn about the various possible combinations of binary organic mixtures

❖ Learn about the detection of hetero elements present in organic compounds.

❖ Know about the systematic procedure for identification of organic compound.

❖ Know how to determine the melting and boiling point of organic compounds.

4.2 SEPARATION OF ORGANIC MIXTURES

Separation of organic mixture is one of the important steps in organic synthesis. Organic mixture can be separated by physical as well as chemical methods. Separations of organic mixture through physical methods include distillation, chromatography, and electrophoresis and counter current separation. In the laboratory the separation of organic compounds present in an organic mixture is generally achieved by their chemical properties, i.e. whether the compound is neutral, acidic or basic in nature. Similarly, differences in the polarities of the components of the mixtures are also a very good criterion for their separation. In many cases this difference must be induced by some simple transformations usually salt formation. Once the significant difference in polarity is established separation can be effected by steam distillation or other extraction methods.

4.2.1 SEPARATION OF BINARY MIXTURES

Binary mixture is a mixture of two different organic components. The separation of binary mixture depends upon the type of mixture. Generally, three types of binary mixtures are possible.

  (i) Solid-solid   (ii) Solid-liquid   (iii) Liquid-liquid

The solid-solid mixture may be homogeneous or heterogeneous. Similarly, the liquid-liquid mixture may be miscible or immiscible.

Organic components present in the mixture can be separated by the following two methods or the combination of these two methods. These methods are:

 i. Solvent separation
 ii. Separation based on salt formation. The reactivity of the components with aqueous solution of sodium bicarbonate, sodium hydroxide and hydrochloric acid can be used to separate the compounds as acid, phenol, base, neutral etc. Thus mixture can be classified according to the following groups on the basis of their chemical nature:

  (a) Acid-base   (b) Acid-neutral   (c) Base neutral
  (d) Acid-phenol   (e) Base-phenol   (f) Neutral-phenol

Before proceeding for the separation of the mixture, the following preliminary investigation of the mixture is done which may give the primary basic idea of the components of the mixture.

4.2.2 PRELIMINARY TESTS

1. Nature: Whether the components are solid or liquid.

   Solid: Carbohydrates, phenols, acids, amines, amides, anilides etc., may be presents.

   Liquids: Lower aromatic hydrocarbons, ethers, alcohols, ketones, aldehydes, esters etc. may be present.

2. Colour: Colour also indicates the type of compound. For example:

   (a) Yellow: Nitro compounds, idoform, diketones and quinones.

   (b) Orange: *o*-nitrophenol, *o*-nitroaniline etc.

   (c) Blackish: α-Naphthol, α-Naphthylamine etc.

   (d) Pink or pale violet: β-Naphthol, β-Naphthylamine etc.

   (e) Buff or reddish brown: Amines, aminoacids, aminophenols etc.

   (f) Colourless or white: Carbohydrates, carboxylic acids, amides, anilides, esters, aldehydes, ketones, lactones, anhydrides, alcohols, halogens, derivatives of hydrocarbons, ethers, etc.

3. Physical state of components in the mixture:

(i) The mixture is completely solid indicates the mixture is solid+solid

(ii) The mixture is semi solid: cool the mixture in ice bath. If the mixture converts in to solid form then the mixture is semi solid+solid. If the mixture remains semi solid on cooling, the mixture is solid+liquid.

(iii) Solid+liquid mixture is one of the following types:

   (a) Volatile liquid + Solid (soluble)

   (b) Volatile liquid + solid (insoluble)

   (c) Non volatile liquid + solid (soluble)

   (b) Non volatile liquid + solid (insoluble)

Nature of solid-liquid mixture can be known as follows:

Take 0.5 g of the mixture in a clean and dry fusion tube. Place a capillary sealed at one end into it in such a way that the open end is dipped into the mixture. Suspend the fusion tube in a beaker containing boiling water. If strong and continuous evolution of air bubble from the open end of the capillary are observed then in that case one of the component is volatile liquid. If no evolution of air bubbles then this indicates that liquid component is non-volatile liquid.

If the volatile liquid is present, put a small amount of the mixture on a watch glass and heat it on a boiling water bath till all volatile liquid is evaporated. Cool the watch glass. Appearance of the solid indicates that the mixture is volatile liquid+solid.

4. Solubility in water, ether and alcohol

The mixture may be:

(i) Soluble + soluble   (ii) insoluble + insoluble   (iii) soluble + insoluble

(a) If one component is soluble and other is insoluble then that solvent can be used for the separation of components from the mixture.

5. Chemical nature (Acidic, phenolic, basic or neutral) of the components of the mixture

(a) Test for the presence of carboxylic acid in the mixture: Shake 0.05 g of the mixture with 5 mL saturated solution of $NaHCO_3$ solution. Formation of strong effervescence indicates the presence of carboxylic acid as one of the component of the mixture.

(b) Test for the presence of phenolic component in the mixture: Place 0.1 g of mixture in the test tube and add 5 mL of ethyl alcohol in it. Warm the content of the test tube in the water bath and filter the content. Filtrate will contain the phenolic component because almost all the phenolic compounds are either soluble (when the component is solid) or miscible (when the components is liquid) in alcohol.

Add alcoholic $FeCl_3$ in the alcoholic filtrate. Formation of blue to green colour indicates that mixture contains phenolic component.

(c) Test for the presence of basic component in the mixture: Basic component in the mixture can only be possible if nitrogen is present in the mixture. Thus, first test the presence of nitrogen as an element in the mixture. If nitrogen is present then perform the following experiment:
Take 0.5 g of the mixture with 5 mL of 1:1HCl in a test tube.

Shake well and filter or separate two layers

Filtrate
(Aqueous Layer)

Residue or non aqueous layer
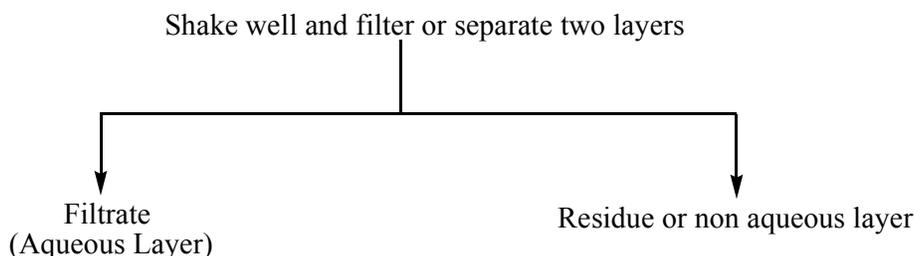
Cool the aqueous layer (filtrate) in an ice bath and add cold 10% NaOH solution drop-wise till the solution becomes alkaline. Cool the resulting alkaline solution in an ice bath.

(i) Appearance of solid: This indicates that basic component is present in the mixture which is in the form of solid.

(ii) Appearance of emulsion: This indicates the presence of basic component in the mixture as a non volatile liquid. Keep the solution for some time; basic compound will separate as an oily liquid.

(iii) No appearance of solid or no emulsion: This indicates that mixture does not contain basic component.

Note: By carrying out of the entire above test if only one component is detected, then the other component must be neutral.

4.2.3 SEPARATION OF SOLID-SOLID MIXTURE

4.2.3.1 SEPARATION BASED ON SOLUBILITY:

In this method one of the components is soluble in the given solvent and the other component remains insoluble. Following are the solvents which are being frequently used for the separation purpose in order of preference.

   i. Water
  ii. Ether
 iii. Absolute alcohol or alcohol

Other organic solvents such as benzene, chloroform and ethyl acetate may also be used as per the requirement. To identify the suitable solvent for separation, a small amount (0.1-0.2g) of the organic mixture is dissolved in the selected solvent. The important observation at this stage is to confirm the complete separation i.e. each constituent must be pure. The purity of the constituents is checked by their melting points. Once a proper selection of the solvent is done an amount of 10 g of the organic mixture may be used for getting separated two components in good amount.

The scheme used for separation is given below:

(A) Separation by water:

Take 0.2g of organic mixture shake it with 10 mL of distilled water in a boiling tube for few minutes and filter the solution	
Residue: Wash the residue with water and dry. Test the purity of the solid. (Component 1)	**Filtrate**: Evaporate the solvent in a watch glass on a boiling water bath. If the residue is obtained then the mixture is separable by water. Test the purity of the solid. (Component 2).

If both the components are pure then repeat the process with 10g of mixture obtained the both compounds in good yield.

If no residue is obtained on evaporation of the filtrate then shake the mixture with hot water and filter in hot condition. Observe whether the mixture is separated by hot water or not.

(B) Separation by organic solvent (say ether): If the mixture is not separated by water then take ether as a solvent and see whether mixture is separated or not as per given procedure:

Take 0.2 g of organic mixture shake it with 10 mL of ether in a boiling tube for few minutes and filter the solution	
Residue: Wash the residue with ether and dry. Test the purity of the solid. (Component 1)	**Filtrate**: Evaporate the solvent in a watch glass on a boiling water bath. If the residue is obtained then the mixture is separable by ether. Test the purity of the solid. (Component 2).

If no residue is obtained on evaporation of the filtrate then ether will not be the suitable solvent for separation. Then try for other solvent(s) as above.

Note: Although glucose, fructose, sucrose, mannose, galactose and other sugars are water soluble, but they yield syrupy liquid, from which it is very difficult to separate them in solid form, therefore, the mixture containing carbohydrate is separated by ether or by absolute alcohol, because carbohydrates are insoluble in ether or absolute alcohol.

4.2.3.2 SEPARATION BASED ON SALT FORMATION:

Separation based on salt formation can only be used if one of the components of the mixture is acidic, phenolic or basic in nature. The salt of the compounds are polar in nature hence soluble in water. This method is highly preferable for the separation of mixture when both components are insoluble in water.

Three cases are possible for the separation of mixture by salt formation.

Case 1: When one of the components in acidic in nature the other component may be phenolic, basic or neutral.

Take 10 g of mixture in a 250 mL beaker. Add 25 mL of saturated sodium bicarbonate solution to it. Stir the content for few minutes till the effervescence stops then filter it:	
Residue: Wash the residue with first with saturated sodium bicarbonate solution to remove all the traces of acid, and then wash with cold water. Dry the residue. The solid thus obtained is component 1. This compound is phenolic, basic or neutral compound. Identify it by usual methods given in the later part of this chapter.	**Filtrate (RCOONa)**: Collect the filtrate in a beaker and cool it in the ice bath. Acidify the content by adding concentrated HCl dropwise (keep on checking with litmus paper) and cool the resulting solution in an ice bath. i. Appearance of solid: This is compound 2; which is a carboxylic acid and it is water insoluble. ii. If compound does not precipitate, collect the solution in a beaker or porcelain dish. Heat it carefully on a wire gauge till the volume of filtrate reduced to one-fourth of its original volume. Cool it, filter it and dry it, the solid obtained is compound 2. iii. If acid still does not precipitate, after concentration, extract the concentrated solution with 10 mL of ether in the separatory funnel and separate the ether layer. Evaporate ether on boiling water bath. The residue left on watch glass is a carboxylic acid which is soluble in water. Crystalline the solid and check the melting point for purity. Identify the compound by usual methods given in the later part of this chapter.

Case II: When one of the components is phenolic and other component is either basic or acidic.

Take 10 g of mixture in a 250 mL of beaker. Add 25 mL of dilute sodium hydroxide solution to it. Stir the content for few minutes then filter it:

| **Residue (basic or neutral compound):** Wash the residue with first with dilute sodium hydroxide solution to remove all the traces of phenolic compound, and then wash with cold water. Dry the residue. The solid thus obtained is component 1. Crystallise the compound and determine its melting point for purity. Identify it by usual methods given in the later part of this chapter. | **Filtrate**: Collect the filtrate in a beaker. Acidify the content by adding concentrated HCl dropwise (keep on checking with litmus paper) and cool the resulting solution in an ice bath.

i.   Phenolic compound obtained as precipitate. Phenolic compound is water insoluble. Wash it with water and dry it. This is compound 2. Crystallize the compound and determine its melting point for purity. Identify it by usual methods given in the later part of this chapter.

ii. If compound does not precipitate, collect the solution in a porcelain dish. Heat it carefully on a wire gauge till the volume of filtrate reduced to one-fourth of its original volume. Cool it, filter it and dry it, the solid obtained is compound 2.

iii. If phenolic compound still does not precipitate, after concentration, extract the concentrated solution with 10 mL of ether in the separatory funnel and separate the ether layer. Evaporate ether on boiling water bath. The residue left on watch glass is a phenolic compound which is soluble in water. Crystalline the solid and check the melting point for purity. Identify the compound by usual methods given in the later part of this chapter. |

Case III: When one of the components is basic; and the other component may be phenolic, acidic or neutral.

Take 10 g of mixture in a 250 mL of beaker. Add 25 mL of 1:1 HCl solution to it. Stir the content for few minutes then filter it:

| **Residue (acidic, phenolic or neutral compound):** Wash the residue with first with | **Filtrate**: Collect the filtrate in a beaker. Basify the content by adding dil. NaOH solution dropwise (keep on checking with litmus paper) and cool the resulting solution in an ice bath.

i.   **Basic compound** obtained as precipitate. Phenolic compound |

1:1 HCl solution, and then wash with cold water. Dry the residue. The solid thus obtained is component 1. Crystallise the compound and determine its melting point for purity. Identify it by usual methods given in the later part of this chapter.	is water insoluble. Wash it with water and dry it. This is compound 2. Crystallize the compound and determine its melting point for purity. Identify it by usual methods given in the later part of this chapter.
	ii. If compound does not precipitate, collect the solution in a porcelain dish. Heat it carefully on a wire gauge till the volume of filtrate reduced to one-fourth of its original volume. Cool it, filter it and dry it, the solid obtained is compound 2.
	iii. If basic compound still does not precipitate, after concentration, extract the concentrated solution with 10 mL of ether in the separatory funnel and separate the ether layer. Evaporate ether on boiling water bath. The residue left on watch glass is a phenolic compound which is soluble in water. Crystalline the solid and check the melting point for purity. Identify the compound by usual methods given in the later part of this chapter.

4.2.4 SEPARATION OF VOLATILE LIQUID-SOLID MIXTURE

Components of such types of mixture are separated by distillation method and distillation is done on a boiling water bath because volatile liquids distil at or below 80°C,

Take 10 g of the mixture (semi solid or liquid mixture) in a 100 mL round bottom flask. Add few porcelain pieces to the flask to prevent bumping if the mixture is in liquid state. Attach a water condenser. Heat the flask on a boiling water bath. The volatile liquid is collected in receiver. When small volume is left in the distillation flask, stop the heating and disconnect the apparatus. The hot residual liquid left in the distillation flask is immediately poured into a dry watch glass. After a few minutes a solid compound is obtained which is collected. It is dried purified and then is used for identification.

4.2.5 SEPARATION OF NON-VOLATILE LIQUID-SOLID MIXTURE

Separation of non-volatile liquid-solid mixture is done by chemical separation using separatory funnel and $NaHCO_3$, NaOH or HCl. Although fractional distillation method can be used for separation but this method is not very useful on small scale.

Three cases are possible in the separation by chemical method.

Case I: When one of the components is acidic.

Take 10 mL of organic mixture in 250 mL of beaker and add 25 mL of saturated sodium bicarbonate solution to it. Stir the content till the effervescence stops. The resulting solution nay contain any one of the following:

   i.   Solid + aqueous layer: This indicates that the non acidic component is solid and acidic component is liquid.

   ii.   Non aqueous layer and aqueous layer: This indicates that acidic component is solid and non-acidic component is liquid

A) When resulting organic mixture contains solid + aqueous layer. Filter the content.

Residue: Wash the residue with sodium bicarbonate solution, and then wash with cold water. Dry the residue. The solid thus obtained is component 1. Crystallise the compound and determine its melting point for purity. Identify it by usual methods given in the later part of this chapter.	**Filtrate**: Take the filtrate in a 100 mL separatory funnel and acidify it by adding 1:1 HCl acid (dropwise keep on checking with litmus paper) and cool the resulting solution in an ice bath. Compound 2 is separated as an oily layer. Separate it from the aqueous layer. The non-aqueous liquid is compound 2. Identify it by usual methods given in the later part of this chapter.

B) When resulting organic mixture contains non-aqueous layer and aqueous layer. Transfer the mixture solution in the 100 mL separatory funnel and separate both the layers

Non-aqueous layer or oily layer: Wash the oily layer with sodium bicarbonate in the separatory funnel and separate the non-aqueous layer.	**Aqueous layer:** Collect the solution in a beaker and cool it in the ice bath. Acidify the filtrate by adding concentrated HCl acid dropwise. Cool the resulting solution in an ice bath. Carboxylic acid separates as

| This is compound 1, which is non-acidic and non-volatile in nature. Identify it by usual methods given in the later part of this chapter. | precipitate. Wash the precipitate with water and dry it. This is compound 2. Identify it by usual methods given in the later part of this chapter. |

Case II: When one of the components is phenolic in nature

Take 10g (or 10 mL) of organic mixture in 250 mL beaker and add 25 mL of dil NaOH solution to it. Stir the content. The resulting solution nay contain any one of the following:

  i.   Solid + aqueous layer: This indicates that the non phenolic component is solid and phenolic component is non-volatile liquid.

  ii.   Non aqueous layer and aqueous layer: This indicates that non-phenolic component is liquid and phenolic component is solid

A) When resulting organic mixture contains solid + aqueous layer. Filter the content.

| **Residue:** Wash the residue with NaOH solution, and then wash with cold water. Dry the residue. The solid thus obtained is component 1. Crystallise the compound and determine its melting point for purity. Identify it by usual methods given in the later part of this chapter. | **Filtrate**: Take the filtrate in a 100 mL separatory funnel and acidify it by adding 1:1 HCl acid (dropwise keep on checking with litmus paper) and cool the resulting solution in an ice bath. Compound 2 is separates as an oily layer. Separate it from the aqueous layer. The non-aqueous liquid is compound 2. Identify it by usual methods given in the later part of this chapter. |

B) When resulting organic mixture contains non-aqueous layer and aqueous layer. Transfer the mixture solution in the 100 mL separatory funnel and separate both the layers

| **Non-aqueous layer or oily layer:** Wash the oily layer with dil NaOH solution in the separatory funnel and separate the non-aqueous layer in test tube. This is compound 1, which is non-phenolic and non-volatile in nature. Identify it by usual methods given in the later part of this chapter. | **Aqueous layer:** Collect the solution in a beaker and cool it in the ice bath. Acidify the filtrate by adding concentrated 1:1 HCl acid dropwise. Cool the resulting solution in an ice bath. Phenolic compound separates as precipitate. Wash the precipitate with water and dry it. This is compound 2. Identify it by usual methods given in the later part of this chapter. |

Case III: When one of the components is basic

Take 10g (or 10 mL) of organic mixture in 250 mL beaker and add 25 mL of dil 1:1 HCl solution to it. Stir the content. The resulting solution may contain any one of the following:

i.   Solid + aqueous layer: This indicates that the non basic component is solid and basic component is non-volatile liquid.

ii.   Non aqueous layer and aqueous layer: This indicates that non-basic component is liquid and basic component is solid

A) When resulting organic mixture contains solid + aqueous layer. Filter the content.

Residue:. Dry the residue. The solid thus obtained is component 1. Crystallise the compound and determine its melting point for purity. Identify it by usual methods given in the later part of this chapter.	**Filtrate**: Take the filtrate in a 150 mL separatory funnel and basify it by adding dil NaOH solution (dropwise keep on checking with litmus paper) and cool the resulting solution in an ice bath. Compound 2 is separates as an oily liquid. Separate it from the aqueous layer. The non-aqueous liquid is compound 2. Identify it by usual methods given in the later part of this chapter.

B) When resulting organic mixture contains non-aqueous layer and aqueous layer. Transfer the mixture solution in the 150 mL separatory funnel and separate both the layers

Non-aqueous layer or oily layer: Wash the oily layer with dil NaOH solution in the separatory funnel and separate the non-aqueous layer in test tube. This is compound 1, which is non-basic and non-volatile in nature. Identify it by usual methods given in the later part of this chapter.	**Aqueous layer:** Collect the solution in a beaker and cool it in the ice bath. basify it by adding dil NaOH solution (dropwise keep on checking with litmus paper). Cool the resulting solution in an ice bath. basic compound separates as precipitate. Wash the precipitate with water and dry it. This is compound 2. Identify it by usual methods given in the later part of this chapter.

4.3 IDENTIFICATION OF ORGANIC COMPOUNDS

4.3.1 PRELIMINARY EXAMINATION

Physical State, Colour and Odour: A careful observation of the physical state, colour and characteristic smell of the compound gives useful information regarding the nature of the compound. For example, the colour of a compound is related to its chemical constitution or else to some impurities in it. Similarly, there are certain groups of compounds which have similar smell. The following table is helpful to some extent in correlating the physical characteristics and the chemical nature.

Colourless solids:	Carbohydrates, simple acids, some phenols, amides and anilides
Coloured solids:	Nitro compounds, amines, phenols, quinines.
Colourless liquids:	Alcohols, aldehydes, ketones, simple hydrocarbons and simple acids.
Coloured liquids :	Nitro compounds, phenols and amines.
Carbolic smell:	Phenols.
Smell of bitter almonds:	Benzaldehyde and nitrobenzene.
Fruity-pleasant smell:	Esters.
Spirituous smell:	Alcohols.
Pungent smell:	Formic acid or formalin.

4.3.2 IGNITION TEST

Take a small portion of the given compound on a metallic spatula and ignite it over non-luminous flame and note the changes:

Observation	Inference
(a) *Burns with a sooty flame*:	May be an aromatic compound.
(b) *Yellow and non-sooty flame*:	May be an aliphatic compound.
(c) *Chars* :	May be a carbohydrate, hydroxyl acid or Sulphanic acids

(d)*Smell of ammonia*:   Nitrogenous compound like urea.

Note: Some of the aliphatic compounds like $CHCl_3$, CCl_4 and C_2Cl_6, owing to high percentage of carbon, give sooty flame.

4.3.3 SOLUBILITY BEHAVIOUR

The behaviour of the compound towards various solvents like water, dilute caustic soda, dil. HCl and conc. H_2SO_4 also reveals its nature. Take a small portion of the substance and note its solubility in above solvents:

Observation	Inference
(a) *Soluble in hot water and solution is acidic to litmus*	Salts of aromatic bases. Lower aliphatic acids, hydroxyl acids or polyhydroxy phenols.
(b) *Soluble in hot water and solution is acidic*	Higher acids or some phenols.
(c) *Soluble in cold water and solution in neutral*	Carbohydrates or alcohols.
(d) *Soluble in hot water and solution is neutral*	Starch, quinones.
(e) *Soluble in cold dil. $NaHCO_3$ with effervescence*	Carboxylic acids.
(f) *Soluble in cold dil. NaOH*	Carboxylic acids or phenols.
(g) *Soluble in hot NaOH and give off ammonia*	Amides, ammonium salts.
(h) *Soluble in cold conc. H_2SO_4*	Aromatic hydrocarbons or phenols.
(i) *Soluble in hot conc. H_2SO_4 and charring occurs*	Carbohydrates, Aldehyde, ketones or hydroxyl acids.
(j) *Soluble in dil. HCl*	Amines

4.3.4 TEST FOR UNSATURATION

(a)   Test with Bromine: Take a solution or suspension of the compounds in water and add to it few drops of bromine water. You may also take a solution/suspension of the compound in carbon tetrachloride and add to it a solution of bromine, shake well and note the changes:

	Observation		**Inference**
(i)	*Decolourisation*	:	Unsaturated compounds containing Alkene(C=C).
(ii)	*A precipitate is obtained*	:	Amines and phenols.

(b)   Test with alkaline KMnO$_4$ solution: Dissolve a small amount of the substance in water or acetone. Add to it 1 ml of dil. sodium carbonate solution and a few drops of potassium permanganate solution and shake well.

If the colour of the permanganate disappears, the given compound may be unsaturated. Heating is forbidden in this test.

4.3.5 DETECTION OF ELEMENTS

All organic compounds contain carbon. In addition, they may contain hydrogen, oxygen, nitrogen, sulphur and halogens. No attempt is made to detect the presence of carbon, hydrogen and oxygen; their presence is usually inferred from chemical reactions. The detection of nitrogen, sulphur and halogens is carried out by converting them into water soluble ionic compounds. This has to be done very carefully because further analysis of the organic compound is largely based on the type of the element present in it.

Lassaigne's test (sodium extract): Nitrogen, sulphur and halogens are usually detected by *Lassaigne's test*. In this test, these elements are converted to water soluble sodium compounds upon fusion with metallic sodium.

In actual procedure, a freshly cut small piece of sodium is first dried by pressing between the folds of filter paper and then put into an ignition tube. The ignition tube is heated very gently until sodium melts. The ignition tube is removed from the flame and a small quantity, of the size of a rice-grain (or 2-3 drops if liquid) of the given compound is put inside it so that it may come

in contact with the molten sodium. Heating is again continued first very gently and then strongly until the bottom of the tube is red hot. It is then plunged into about 10 mL of distilled water contained in a beaker or porcelain dish. The ignition tube if not broken completely, is crushed with the help of a glass rod. The contents in the dish are stirred with glass rod, boiled for about five minutes and filtered. The filtrate is called sodium extract with which the qualitative tests for nitrogen, sulphur and halogens are performed as ahead. The filtrate should be water clean and alkaline. If it is dark in colour, it indicates incomplete fusion and the process is repeated with fresh ignition tube.

1. *Test for nitrogen***:**

Take about 2-3 ml of sodium extract (fusion solution) in a test tube and add to it 0.1 – 0.2 g of powdered ferrous sulphate. This gives a dirty green precipitate of ferrous hydroxide. If no precipitate is formed, add a few drops of dilute sodium hydroxide solution to get the precipitate. Now heat this mixture gently with shaking to boiling and without cooling, add dilute sulphuric acid to dissolve the precipitate and add some more to make the solution acidic. *A Prussian blue colour or precipitate confirms the presence of nitrogen*. If the colour or precipitate does not appear at once allow it to stand for ten minutes to obtain so. In absence of nitrogen, the solution will remain pale yellow due to the presence of iron compound.

2. *Test for sulphur***:**

(i)   To about 1 mL of sodium extract, add 1 to 1.5 mL of freshly prepared sodium nitroprusside solution. If a purple colour is obtained, sulphur is present. This colour fades away slowly.

(ii)   Acidify 1 mL of sodium extract with excess of acetic acid and then add a few drops of lead acetate solution. A black precipitate indicates the presence of sulphur.

3. *Test for halogens***:**

After ascertaining the presence or absence of nitrogen and sulphur, test for halogens should be performed. For it two separate procedures may be adopted:

(a)   When *nitrogen and sulphur are absent.* Acidify 1-2 mL of sodium extract with dilute nitric acid and to it add 2-3 mL of silver nitrate solution and observe:

(i) A white precipitate soluble in NH_4OH indicates the presence of chlorine.

(ii)A pale yellow precipitate, sparingly soluble or soluble in excess of NH_4OH indicates the presence of bromine.

(iii) A yellow precipitate insoluble in NH_4OH indicates the presence of iodine.

Bromine and Iodine.   Acidify 1-2 mL of sodium extract with dilute HNO_3 and 1 mL of chloroform and excess of chlorine water and shake well. If the chloroform layer becomes yellow or brown, bromine is present. If the layer turns violet, presence of iodine is indicated.

(b)   *When nitrogen and/or sulphur present.* Take about 2-3 mL of sodium extract and acidify with some dilute nitric acid. Now evaporate the solution to almost 1 mL. This will expel hydrogen cyanide and hydrogen sulphide. Dilute the solution with equal volume of water and test for halogens as given in part (a).

Chemistry involved in Lassaigne's test:

When an organic compound is fused with sodium, the nitrogen of the compound is converted into sodium cyanide, sulphur into sodium sulphide and halogens into sodium halides. These sodium salts being soluble in water come into aqueous solution. These salts are ionisable and hence the presence of cyanide, sulphide or halide ions is easily detected through the usual tests.

$$\text{Na } + \text{C} + \text{ N} \longrightarrow \underset{\text{Sodium cyanide}}{\text{NaCN}}$$

$$\text{2 Na } + \text{S} \longrightarrow \underset{\text{Sodium sulphide}}{\text{Na}_2\text{S}}$$

$$\text{Na } + \text{ X} \longrightarrow \underset{\substack{\text{Sodium halide} \\ \text{(X = Cl, Br, I)}}}{\text{NaX}}$$
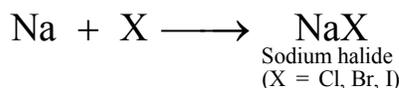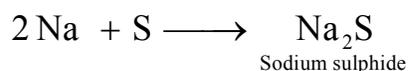
When nitrogen and sulphur both are present, sodium thiocyanate is formed.

$$\text{Na } + \text{C} + \text{ N } + \text{ S} \longrightarrow \text{NaCNS}$$

In the test for nitrogen, when ferrous sulphate is added to sodium extract, it reacts with the alkali (produced by reaction between Na and H_2O) and dirty green precipitate of ferrous hydroxide is produced. This reacts with sodium cyanide to give sodium ferrous cyanide which with ferric ion produces bluish ferriferrocyanide.

$$FeSO_4 \ + \ 2\,NaOH \longrightarrow \underset{\substack{\text{Ferrous hydroxide} \\ \text{(dirty green)}}}{Fe(OH)_2} \ + \ Na_2SO_4$$

$$Fe(OH)_2 \ + \ 3\,NaCN \longrightarrow \underset{\text{Sodium ferrocyanide}}{Na_4Fe(CN)_6} \ + \ 2\,NaOH$$

$$3\,Na_4Fe(CN)_6 \ + \ 4\,FeCl_3 \longrightarrow \underset{\substack{\text{Ferr-ferrocyanide} \\ \text{(Prussian blue)}}}{Fe_4[Fe(CN)_6]_3} \ + \ 12\,NaCl$$

In the test for sulphur, a violet coloured complex is produced upon addition of sodium nitroprusside to the sodium extract.

$$Na_2S \ + \ \underset{\text{Sodium nitroprusside}}{Na_2[Fe(NO)(CN)_5]} \longrightarrow \underset{\text{Purple coloured complex}}{Na_3[Fe(O=N-S-Na)(CN)_5]}$$

When the sodium extract is treated with lead acetate, a black precipitate of lead sulphide insoluble in acetic acid is produced.

$$Na_2S \ + \ (CH_3COO)_2Pb \longrightarrow \underset{\text{Black precipitate}}{PbS} \ + \ 2\,CH_3COONa$$

In the test for halogens, when sodium extract is boiled with conc. HNO_3, sodium cyanide, if present, is decomposed and is eliminated as HCN gas. If NaCN is not decomposed, it gives white precipitate of $AgNO_3$ even in absence of halogens hence it is confusing.

After decomposing NaCN, $AgNO_3$ is added thereby silver halide is precipitated.

$$NaCN \ + \ HNO_3 \longrightarrow NaNO_3 \ + \ HCN$$

$$NaX \ + \ AgNO_3 \longrightarrow \underset{\text{precipitate}}{AgX} \ + \ NaNO_3$$

The chloroform test for bromine and iodine is based on the principle that chlorine liberates bromine and iodine from sodium bromide and sodium iodide. Free bromine and iodine are

soluble in chloroform, therefore, chloroform layer becomes yellow if bromine is present and it becomes violet if there is iodine.

4.3.6 DETECTION OF FUNCTIONAL GROUPS

The determination of functional groups depends largely on the correct determination of elements. Once the functional group present in the organic compound is known, one is able to find out the name of the probable compound with the help of melting point or boiling point. Next procedure is to study some specific reactions of the compound and then to confirm its name by preparing suitable derivatives. It may be noted that element carbon, hydrogen and oxygen are not tested for. This clearly means that if not other element is present, functional group containing carbon, hydrogen and oxygen are determined.

The test for functional groups should be performed according to the following way:

[A]   When only Carbon, Hydrogen and/or Oxygen are present

S. No.	Class	Functional group	Tests
1.	Carboxylic acid	$-C{\Large\langle}^{O}_{OH}$	(i) **Litmus test.** Shake a small amount of the given substance in water and dip a blue litmus paper in it. If the litmus paper turns red, it shows presence of carboxylic group (phenols being acidic in nature also give this test).
			(ii) **Sodium bicarbonate test.** Add a pinch of the given substance if solid (or a few drops if liquid) to 5 mL of cold 50% solution (aqueous) of $NaHCO_3$ and shake gently. Effervescence (or slow up-and-down movement of particles) indicates presence of carboxylic group.
2.	Phenol	Phenolic-OH	(i) Blue litmus changes to red but no effervescence with $NaHCO_3$.
			(ii) **FeCl₃ test.** To 1 mL of aqueous or alcoholic solution of original substance (O.S) add 2-3

			drops of neutral or very dilute solution (aqueous) of ferric chloric. A blue, green, red or violet colour indicates presence of phenolic group $(-OH)$. (iii) **Libermann's test.** All phenols in which para position is free respond to this test. Take 0.2 g of O.S. and a few crystals of $NaNO_2$ in a dry test tube and heat gently for a minute. Cool the mixture, add 1 mL of conc. H_2SO_4 and shake. A deep green or blue colour develops, upon dilution with water the solution turns red. To it, add excess of dil. NaOH solutions, the red solution again becomes deep green or blue. It indicates the presence of phenolic group. (iv) **Phthalein test.** Take 0.2 g each of O.S. and phthalic anhydride in a dry test tube, add 0.5 mL of conc. H_2SO_4 and heat for a minute. Cool, make it alkaline with dil. NaOH solution. Pour a few drops of this solution in 20 mL water taken in a beaker. Characteristic colour shows the presence of phenolic group, e.g. Pink colour – Phenol, *o*-Cresol Blue colour – Catechol, *m*-Cresol NO colouration – *p*-Cresol.
3.	Carbohydrate	……………	(i) **Blackening with H_2SO_4.** Heat 0.2 g of O.S. with 1 mL conc. H_2SO_4. An immediate charring and blackening of the solution shows presence of carbohydrate. (ii) **Molisch's test.** To 2 mL aq. solution of O.S. add 1 mL of Molisch's reagent and shake well. Now add carefully 1-2 mL of conc. H_2SO_4

				(taken in a separate test tube) from the side of the test tube. Allow to stand. A reddish violet ring at the junction of two liquids shows the presence of carbohydrate.
			(iii)	To 1 mL aq. solution of O.S. add 1-2 drops of anthrone solution shake and warm the mixture gently. A green or bluish green colour shows the presence of carbohydrate.
4.	Aldehydes	$-C{\Large\diagup}^{O}_{\diagdown H}$	**(i)**	**Schiff Test.** To 1 mL aq. or alcoholic solution of O.S. and 2 mL of Schiff reagent and shake. A deep red or violet colour shows the presence of aldehydic group (never heat during this test).
			(ii)	**Tollen's test.** To about 2 mL of Tollen's reagent add about 0.5 g of O.S. and heat the mixture on water bath. Formation of silver mirror or blackish precipitate indicates the presence of aldehydic group. (This test is also given by reducing sugars).
			(iii)	**Fehling solution test.** Add Fehling solution B to A until a blue precipitate first formed is redissolved to give a deep blue solution. To it add a little of O.S. and heat for 2 minutes. Reddish brown precipitate shows the presence of aldehydic group. (This test is also given by reducing sugars).
5.	Ketones	$\diagup \!\!\!\! {}_{\diagdown}C{=}O$	**(i)**	No colour with Schiff reagent.
			(ii)	**2, 4 Dinitrophenylhydrazine test.** Add 1-2 drops (0.5 g) of O.S. to 2 mL of 2, 4 dinitrophenylhydrazine solution (Brady's reagent). Shake vigorously. Heat and cool. A red, yellow or orange. Coloured precipitate

			indicates the presence of ketonic group.
6.	Esters	—C≪O, OR	**(i) Hydroxamic acid test (Feigl test).** Take a rice grain (R.G.) or two drops of the compound in a test tube, add 1 mL of 5% methanolic solution of hydroxylamine hydrochloride. Now, add a few drops of 2N methanolic KOH until the solutions is just alkaline. Boil, cool and acidify with dil. HCl solution. If Deep red or violet colour is produced, it indicates the presence of ester group. **(ii) Hydrolysis test.** Dissolve 0.5 g of O.S. in 2 mL alcohol. To it, add 4-5 drops of dil. NaOH solution and 2-3 drops of phenolphthalein. A pink colour is produced. Now put the test tube in boiling water bath for 5 minutes. If the pink colour either fades or disappears, this shows presence of ester group.
7.	Alcohols	$-OH$	**(i)** In a dry test tube, take 2 mL of O.S, add 1 g of anhydrous sodium sulphate (to absorb water if already present) and filter. To the filtrate, add a small piece of sodium. Effervescence due to evolution of H_2 shows the presence of an alcohol. **(ii)** To 1 mL of O.S. or its aqueous solution, add a few drops of cerric ammonium nitrate. A pink or red colour shows the presence of an alcohol.
8.	Hydrocarbons	Nil	No specific group test.

[B]   When nitrogen is present

S. No.	Class	Functional group	Tests
1.	Amides	$-C\diagup^{O}_{\diagdown NH}$	To 0.2 g or 0.5 mL of O.S., add 1 mL of aqueous NaOH and heat. Smell of ammonia shows the presence of an amide.
2.	Primary amines	$-NH_2$	**(i) Carbylamine Test.** Take a small amount of substance, 4 mL of alcoholic solution of KOH and 2 drops of chloroform in a test tube, shake and heat gently. An intolerable offensive odour of carbylamine shows the presence of primary amine. **(ii) Nitrous acid test.** Take 0.2 g of O.S. in 2 mL of dil. HCl and cool. Now add 10% aq. $NaNO_2$ solution. A brisk effervescence shows the presence of an aliphatic primary amine. **(iii) Diazotisation Test.** Dissolve 0.2 g or 0.5 mL of O.S. in 2-3 mL of dil. HCl. Cool under tap water. Now add 2 mL of 2.5% $NaNO_2$ solution (aq.), cool again and add 0.5 mL of alkaline β - naphthol solution. An orange-red or a red dye shows the presence of **aromatic** primary amine.
3.	Secondary amines	$>NH$	**(i)** Do not give carbylamines test. **(ii) Nitrous acid test.** Take 0.2 g or 0.5 ml of O.S., add 2 mL of dil. HCl and cool under tap water. Add 2 mL of 2.5% $NaNO_2$ solution. Yellow oily drops are obtained. This shows the presence of secondary amine. (This test is given by both, aliphatic and aromatic secondary amines). **(iii) Libermann's nitroso reaction.** Take 0.2 g or 0.5 mL of O.S., add 2 mL of dil. HCl and cool

			under tap water. Add 2.5% aqueous solution of NaNO$_2$ gradually with shaking until yellow oil separates at the bottom. Decant off the aqueous layer. Now, take 2 drops in test tube, and add 0.5 g of phenol and warm gently for a few seconds. Cool and add 1 mL of conc. H$_2$SO$_4$. A greenish blue colour is obtained which changes red upon dilution with water. On adding excess of NaOH solution, the greenish blue colour is restored. This shows the presence of secondary amine.
4.	Tertiary amines	$>$N:	**(i)** Do not give carbylamine test. **(ii)** Dissolve 0.2 g or 0.5 mL of O.S. in 2-5 mL of dil. HCl and cool under tap water. Add 2-5 mL of 2.5% aqueous solution of NaNO$_2$ gradually with shaking and observer: (a) No reaction indicates the presence of aliphatic tertiary amine (b) Production of green or brown coloured salt indicates the presence of aromatic tertiary amine.
5.	Anilides	ArNHCOR	**(i) Carbylamine test.** Heat a mixture of 0.5 g of O.S., 4 mL of alcoholic KOH solution and 1 mL chloroform. Bad smell of carbylamine comes out. **(ii) Tafel's test.** Take 0.2 g of O.S., add 5 mL of conc. H$_2$SO$_4$ and shake. Add 0.2 g of powdered potassium dichromate. A red or violet colour which changes to green on standing, shows the presence of an anilide. **(iii) Hydrolysis test.** Take 0.2 g of O.S., add 5 mL

			of dil. HCl, boil and cool. Add 2 mL of 2.5% NaNO$_2$ solution, cool again and add 0.5 mL of alkaline β-naphthol solution. An orange-red dye shows the presence of an anilide. (Anilides are hydrolysed on boiling with HCl and thus liberate primary amino group which gives the *dye test*).
6.	Nitro compounds	– NO$_2$	**(i)** **Mulliken-Barker test.** Take 0.2 g or 2-3 drops of O.S. in a test tube, add 4-5 mL of alcohol, a pinch of zinc dust and nearly 10 drops of 10% CaCl$_2$ or NH$_4$Cl solution. Boil and cool. Filter it directly into 2 mL. Tollen's reagent, taken in another test tube. On warming, a grey or black precipitate or silver mirror is produced which confirms the presence of a nitro group. **(ii)** **Azo dye formation.** In a boiling tube, take 0.2 g or 3 drops of O.S., 3 mL conc. HCl, 2 mL water, and 1 g of solid stannous chloride (or tin granules). Heat the contents on water bath for few minutes. Filter and cool. To the filtrate, and 2.5% aq. NaONO$_2$ solution drop by drop to complete the diazotization. Cool again and add 1-2 mL of alkaline β-naphthol solution. An orange-red or red dye shows the presence of nitro group. (The dye is formed due to liberation of primary amino group by reduction of nitro group).

[C]   When nitrogen and sulphur are present

S. No.	Class	Functional group	Tests
1.	Thioureas	—HN—C—NH— ‖ S	(i) Simple thiourea upon boiling with NaOH solution, gives off ammonia. (ii) Boil a little amount of the substance with dil. NaOH solution, cool and add lead acetate. A brown or black colour of precipitate indicates the compound to be a thiourea.
2.	Amino sulphonic acid	$- NH_2$ and $- SO_3$ groups	Dissolve a little of the O.S. in dil. HCl. Cool and add $NaNO_2$ solution so as to complete the diazotization. Now, add alkaline solution of β-naphthol. Red or orange dye is formed.

[D]   When halogen is present

S. No.	Class	Functional group	Tests
1.	Halogenated (a) Aliphatic halgogen compounds, RX	……………	Boil 0.5 mL of O.S. with 5 mL of alcoholic NaOH solution for about 10 minutes. Cool, dilute the solution with water, add excess of dil. HNO_3 and then $AgNO_3$ solution. A precipitate of silver halide is obtained. Test depends on the type of the additional functional group present.
	(b) Aromatic halogen compounds	……………	Test as above

4.3.7 DETERMINATION OF MELTING OR BOILING POINTS

This is the most important step in the identification of an organic compound. Pure organic compounds have fixed melting or boiling points and hence this determination helps to identify the individual compound once the nature of the functional group present in it is ascertained.

(A) Determination of melting point:

The compound whose melting point is to be determined, is powdered thoroughly on a porous plate with the help of a spatula. A capillary tube of approximately 2″ length is sealed at one end by heating in a Bunsen flame. It is then filled up to about 1 cm length with the powdered substance. The capillary is then attached to the lower end of the thermometer as shown in **Fig. 1**. The thermometer is now placed in a Thiele tube filled with paraffin oil or concentrated sulphuric acid such that the liquid covers atleast the filled length of the capillary. Cork used is split one to allow for expansion of air.
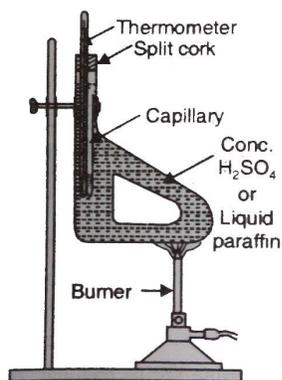

Fig. 1 (Melting point apparatus)   **Fig. 2** (Boiling point apparatus)
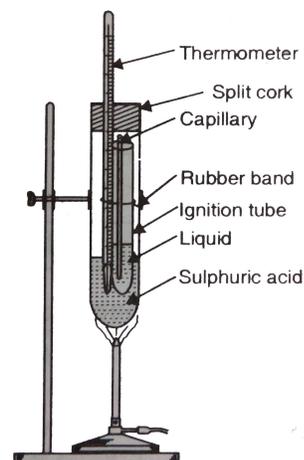
The flask is gently heated from the point shown and rise in temperature is observed carefully. The temperature at which the substance begins to liquefy is noted. The temperature at which the solid has completely changed into liquid is also noted. This range of temperature is recorded as M.P. range of the substance. In case compound is pure, the melting point is quite sharp and occurs over 0.5 to $1^{o}C$ range of variation. During melting of the solid, the temperature remains constant.

(B) Determination of boiling point:

The boiling point of a liquid may be recorded in either of the two ways depending on the availability of the appliances.

(i) First Method: A few drops of the liquid whose boiling point is to be determined is taken in an ignition tube. A capillary tube sealed at the upper end is put inside the ignition tube and the latter is attached to the lower part of the thermometer with the help of a rubber thread. The thermometer along with the ignition tube is placed inside a pyrex test tube in such a way that the liquid inside the ignition tube is covered by concentrated H_2SO_4 taken in the pyrex tube as shown in the **Fig. 2**.

The test tube is heated slowly and the rise of bubbles inside the capillary is carefully observed. The temperature at which a regular and speedy stream of bubbles beings to escape from the surface of liquid is consider as the boiling point of the liquid.

(ii) Second Method: As shown in **Fig. 3**, the liquid whose boiling point is to be determined is taken in a distillation flask. Some pumice stones or porcelain pieces are added to avoid bumping. A thermometer is fitted in the flask through a cork. The delivery tube of the flask is connected to a water condenser. If the condenser is not provided, vapours can be also collected in a test tube.

The flask is heated and rise in temperature is carefully observed. The liquid begins to distil over after sometime. The bulk of the liquid distils over with a certain temperature range which remains nearly constant throughout the distillation. This temperature range is taken to be the boiling point of the liquid.

This method is useful because in addition to determination of the boiling point, one can also purify the given liquid by distillation. Now-a-days, the boiling point is more conveniently determined by test-tube like flask with an outlet at the top from where the vapours are collected in a test tube (**Fig. 4**).
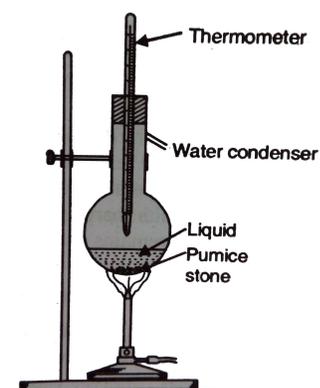
Fig. 3
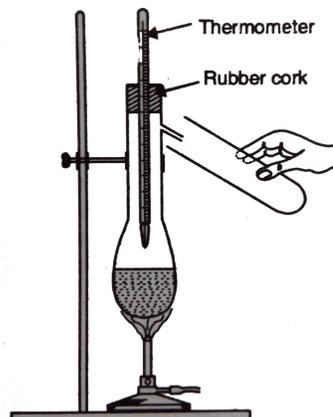
Fig. 4

4.4 SUMMARY

- The present unit comprises the detailed description of systematic separation and identification methods for binary organic mixture.

- Different possible binary combinations of organic compounds and their separation methods are also discussed in the present unit.

- Importance of solubility behavior in separation of binary mixture is also discussed in present unit.

- The unit also comprises how to separate the phenolic, acidic, basic and neutral organic compounds when present together.

- Methods for element detection are also discussed in detail.

- Determination of various functional groups and their confirmatory tests are also included in the unit.

- To check the purity of the organic compound, determination techniques for melting point and boiling point is also a part of this unit.

UNIT 5: QUANTITATIVE AND SPECTROPHOTOMETRIC ESTIMATIONS

CONTENTS:

## 5.1 OBJECTIVES

In present unit students will be able to

   ❖ Estimate the percentage and number of hydroxyl group present in given organic compound

❖ Estimate the percentage and number of amine present in given organic compound using different methods

❖ Estimate the percentage and number of phenol present in given organic compound

❖ Determine the iodine and saponification values of oils and fats.

❖ Determine the dissolved oxygen, biological oxygen demand and chemical oxygen demand in water sample.

❖ Learn the spectrophotometric estimation of various synthetic and natural organic molecules.

5.2 DETERMINATION OF THE PERCENTAGE OR NUMBER OF HYDROXYL GROUPS IN AN ORGANIC COMPOUND BY ACETYLATION METHOD

Theory

The percentage or number of hydroxyl groups in an alcohol or phenol may be determined by acetylation (esterification) with acetic anhydride-pyridine reagent. A known weight of sample (i.e. phenols or alcohols) is acetylated with known volume of acetic anhydride (used in excess) and pyridine. The excess of acetic anhydride is hydrolysed with water to give acetic acid. The left over amount of acetic acid is then determined by titration with standard alkali using phenolphthalein as indicator. This gives the amount of unreacted acetic anhydride. A blank experiment is simultaneously performed without adding the hydroxy compound. The difference between the amounts of alkali used in the two titrations is equivalent to the amount of acetic acid formed from the anhydride used in the acetylation of hydroxyl groups.

$$R(OH)n \quad + \quad n(CH_3CO)_2O \quad \xrightarrow{\text{Pyridine}} \quad R(OCOCH_3)n \quad + \quad nCH_3COOH$$

$$(CH_3CO)_2O \quad + \quad H_2O \quad \xrightarrow{\text{Hydrolysis}} \quad 2CH_3COOH$$

$$CH_3COOH \quad + \quad NaOH \quad \xrightarrow{\text{Neutrilisation}} \quad CH_3COONa \quad + \quad H_2O$$
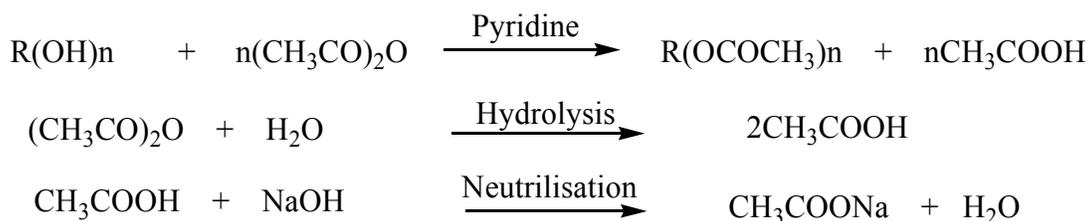
Pyridine acts as solvent and as catalyst in the acetylation reaction as it removes the acid by salt formation. An advantage of using pyridine in this reaction is that, it does not react with acetic anhydride.

$$ROH \quad + \quad (CH_3CO)_2O \quad + C_5H_5N \quad \longrightarrow \quad ROCOCH_3 \quad + \quad C_5H_5NHOOCCH_3$$
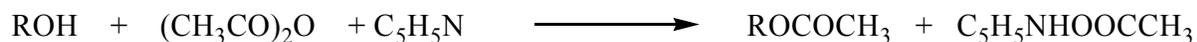
If molecular weight of hydroxyl compound is known then the number or percentage of hydroxyl group can be calculated.

Reagents

1. Acetylating mixture: Thoroughly mix one volume of acetic anhydride with three volumes of pyridine.

2. N/2 alcoholic NaOH solution: Prepare saturated solution of NaOH in water (about 18-20N). Take 7 mL of this solution in a 250 mL of measuring flask and make up it up to 250 mL by adding ethanol or methanol. Standardise this solution with standard N/2 oxallic acid or N/2 sulphuric acid using phenolphthalein as an indicator.

3. Indicator: Phenolphthalein

Procedure:

Take 1-1.5 g of sample in a 100 mL round bottom flask fitted with reflux condenser. Add 10 mL of the acetylating mixture to it and heat the contents in a water bath for about 30 minutes. Allow the flask to cool and then add 20 mL of distilled water through the condenser and shake the flask well. Again heat the flask for 5 minutes, allow it to cool for 15 minutes with occasional shaking to ensure hydrolysis of the remaining acetic anhydride. Remove the condenser and titrate the content (take 5 mL for each run) of the flask with standard N/2 alcoholic NaOH solution using phenolphthalein indicator. Repeat the above titration at least three times. Perform the blank titration without adding the sample of the hydroxyl compound.

Calculation:

Weight of the sample = W g

Volume of NaOH used with the sample = V_1 mL

Volume of NaOH used with the blank = V_2 mL

Normality of NaOH solution = N/x

1000 mL of $1N$ NaOH $\equiv$ 1 g moles of NaOH $\equiv$ 1 g moles of CH_3COOH $\equiv$ 1 g moles of hydroxyl group

therefore (V_2-V_1) mL of N/x NaOH $\equiv \left(\dfrac{V_2 - V_1}{1000}\right) \times \dfrac{N}{x}$ hydroxyl group

since   W g of the hydroxyl compound contains $= \left(\dfrac{V_2 - V_1}{1000}\right) \times \dfrac{N}{x}$ hydroxyl group

therefore 100 g of hydroxyl compound conatins $= \left(\frac{V_2 - V_1}{1000 \times W}\right) \times \frac{N}{x} \times 100$ hydroxyl group

$$= \left(\frac{V_2 - V_1}{10 \times W}\right) \times \frac{N}{x} \text{ hydroxyl group}$$

Thus, the % of the hydroxyl group in the sample of an unknown compound

$$= \left(\frac{V_2 - V_1}{1000 \times W}\right) \times \frac{N}{x}$$

If the molecular weight of the hydroxyl compound is known, suppose it is M, then the number of hydroxyl group present in the compound will be

$$= \left(\frac{V_2 - V_1}{1000 \times W}\right) \times \frac{N}{x} \times M$$

5.3 ESTIMATION OF THE PERCENTAGE OR NUMBER OF AMINES IN AN ORGANIC COMPOUND BY ACETYLATION METHOD

Theory

Amino compounds react with acetic anhydride (known amount) in pyridine at room temperature to give acetyl derivative. The acetylation involves heating of a known amount of amine with known volume of acetic anhydride (used in excess) and pyridine until acetylation is complete. The excess amount of unreacted acetic anhydride present in the reaction mixture is hydrolysed to acetic acid by adding water to it. The total free acetic acid is then titrated with standard alkali solution. Simultaneously a blank experiment is also carried out excluding amino sample. The difference in volume of the alkali required in the two experiments is equivalent to the amount of acetylating agent taken up by the amine.

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

$$(CH_3CO)_2O \quad + \quad H_2O \xrightarrow{\text{Hydrolysis}} 2CH_3COOH$$

$$RNH_2 \quad + \quad (CH_3CO)_2O \xrightarrow{\text{Pyridine}} RNHCOCH_3 \quad + \quad CH_3COOH$$
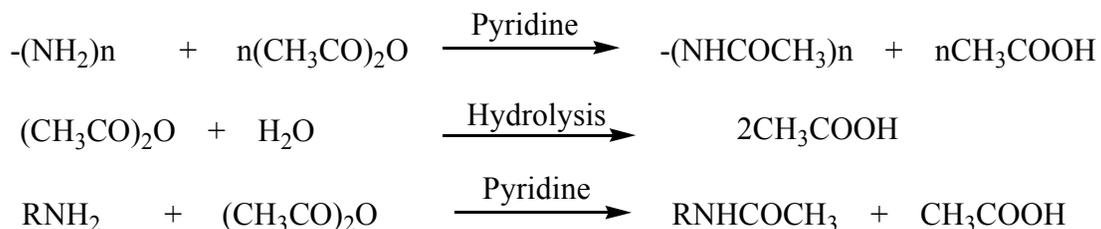
If the molecular weight of amino compound is known then the percentage and number of amino group present in the given compound can be calculated. Various secondary aliphatic, aromatic or heterocyclic amines may be estimated by using this method.

Reagents

1. Acetylating mixture: Thoroughly mix one volume of acetic anhydride with three volumes of pyridine.

2. N/2 alcoholic NaOH solution: Prepare saturated solution of NaOH in water (about 18-20N). Take 7 mL of this solution in a 250 mL of measuring flask and make it up to 250 mL by adding ethanol or methanol. Standardise this solution with standard N/2 oxallic acid or N/2 sulfuric acid using phenolphthalein as an indicator.

3. Indicator: Phenolphthalein

Procedure:

Take 1-1.5 g of sample in a 100 mL round bottom flask fitted with reflux condenser. Add 10 mL of the acetylating mixture to it and heat the contents in a water bath for about 30 minutes. Allow the flask to cool and then add 20 mL of distilled water through the condenser and shake the flask well. Again heat the flask for 5 minutes, allow it to cool for 15 minutes with occasional shaking to ensure hydrolysis of the remaining acetic anhydride. Remove the condenser and titrate the contents (take 5 mL for each run) of the flask with standard N/2 alcoholic NaOH solution using phenolphthalein indicator. Repeat the above titration at least three times. Perform the blank titration without adding the sample of the amino compound.

Calculation:

Weight of the sample $= W$ g

Volume of NaOH used with the sample $= V_1$ mL

Volume of NaOH used with the blank $= V_2$ mL

Normality of NaOH solution $= N/x$

1000 mL of $1N$ NaOH $\equiv$ 1 g moles of NaOH $\equiv$ 1 g moles of $CH_3COOH \equiv$ 1 g moles of amino group

therefore $(V_2\text{-}V_1)$ mL of N/x NaOH $\equiv \left(\frac{V_2 - V_1}{1000}\right) \times \frac{N}{x}$ amino group

since W g of the amino compound contains $= \left(\frac{V_2 - V_1}{1000}\right) \times \frac{N}{x}$ amino group

therefore 100 g of amino compound conatins $= \left(\frac{V_2 - V_1}{1000 \times W}\right) \times \frac{N}{x} \times 100$ amino group

$$= \left(\frac{V_2 - V_1}{10 \times W}\right) \times \frac{N}{x} \text{ amino group}$$

Thus, the % of the amino group in the sample of an unknown compound

$$= \left(\frac{V_2 - V_1}{1000 \times W}\right) \times \frac{N}{x}$$

If the molecular weight of the amino compound is known, suppose it is M, then the number of amino group present in the compound will be

$$= \left(\frac{V_2 - V_1}{1000 \times W}\right) \times \frac{N}{x} \times M$$

5.4 ESTIMATION OF THE AMINES IN AN ORGANIC COMPOUND USING BROMATE-BROMIDE SOLUTION METHOD

Sub object: To determine the amount of aniline in g/lit in its given solution

Theory

Aromatic amines (aniline, benzylamine, m-toludine etc.) having free *ortho* and/or *para* positions can be quantitatively brominated with brominating agent (mixture of $KBrO_3$ + KBr) in the presence of concentrated HCl. Excess of bromine is then determined by adding KI solution to liberate equivalent amount of iodine which is then estimated by titrating against standard solution of sodium thiosulphate (hypo) solution using starch indicator. The number of bromine atoms consumed and time required is dependent upon the structure of amine. For example, aniline generally consumes 6 bromine atoms, *p*-chloroaniline consumes 4 bromine atoms, *m*-toludine consumes 6 and *o*-toludine consumes 4 bromine atoms. The reaction is generally completed in 10-45 minutes.

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

2,4,6-tribromobenzenamine

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

$$I_2 + Na_2S_2O_3 \longrightarrow Na_2S_4O_6 + 2NaI$$
$$\text{(hypo)}$$

Reagents:

1. Brominating mixture (~0.1N): In a 100 mL measuring flask dissolve 0.4 g of anhydrous $KBrO_3$ (potassium bromate) and 1.8 g of KBr (Potassium bromide) in distilled water and make up to 100mL.

2. Sodium thiosulphate solution (approximately 0.1N): Dissolve 6.2 g of sodium thiosulphate in distilled water and make up to 250 mL. For better results, this solution must be prepared one day before the experiment. Standardise the solution idometrically to know its exact normality using standard solution of potassium dichromate ($K_2Cr_2O_7$) or copper sulphate.

3. Potassium iodide solution (20%): Dissolve 15 g of potassium iodide in 75 mL of distilled water.

4. Indicator: 1% starch solution

5. Stock solution of aniline: Stock solution of aniline is generally prepared by dissolving 8 g of aniline in 1000 mL of water containing 1 to 2 mL of concentrated HCl. For experiment 10 mL from the stock solution should be given to each student.

Procedure:

Make up given solution (10 mL) of aniline up to 100 mL in a measuring flask. Pipate out 25 mL of this aniline sample in a 250 mL conical flask with stopper (iodine flask), to it add 25 mL of bromate bromide solution along with 25 mL of distilled water. Add 5 mL of concentrated HCl to the reaction mixture, close the flask with stopper immediately, and shake well to mix the contents. Allow the flask to stand for 10 minutes with occasional shaking. Now add 25 mL of 20% potassium iodide solution to the reaction mixture and shake well.A dark yellow colour is

developed due to the liberation of iodine and its dissolution in KI. Titrate the liberated iodine, which is equivalent to excess of bromine taken, with standard hypo (sodium thiosulphate) solution using starch as indicator. The end point is marked by the disappearance of blue colour. Perform a blank experiment without using aniline solution.

Calculation:

Volume of hypo used with the sample = V_1 mL

Volume of hypo used with the blank = V_2 mL

Normality of hypo solution = N/x

1000 mL of 1N hypo solution ≡ 1 g equivalent weight of hypo ≡ 1 g equivalent weight of Br

∴ (V_2-V_1) mL of N/x hypo solution ≡ $\left(\frac{V_2 - V_1}{1000}\right) \times \frac{N}{x}$ g equivalent weight of hypo or Br

$$= \left(\frac{V_2 - V_1}{1000}\right) \times \frac{N}{x} \times \frac{1}{6} \text{ moles of aniline}$$

Because from the reaction given above, 1 g mole of aniline = 6 g equivalent weight of Br

therefore 25 mL of the aniline sample conatins = $\left(\frac{V_2 - V_1}{1000}\right) \times \frac{N}{x} \times \frac{93}{6}$ g of aniline

(where 93 is the molecular weight of aniline)

Thus, 1 liter of aniline sample contains = $\left(\frac{V_2 - V_1}{1000}\right) \times \frac{N}{x} \times \frac{93}{6} \times \frac{1000}{25}$ g of aniline

$$= \left(\frac{V_2 - V_1}{150}\right) \times \frac{N}{x} \times 93 \text{ g of aniline}$$

Amount of aniline g/liter will be

$$= \left(\frac{V_2 - V_1}{150}\right) \times \frac{N}{x} \times 93$$

5.5 ESTIMATION OF PHENOLS IN AN ORGANIC COMPOUND USING BROMATE-BROMIDE SOLUTION METHOD

Sub object: To determine the amount of phenol in g/lit in its given solution

Theory

The procedure is quite similar to the procedure followed for the estimation of amine in an organic compound. Phenols (e.g. phenol, cresols, di- and trihydric phenols etc.) having free *ortho* and/or *para* positions can be quantitatively brominated with brominating agent (mixture of $KBrO_3$ + KBr) in the presence of concentrated HCl. Excess of bromine is then determined by

adding KI solution to liberate equivalent amount of iodine which is then estimated by titrating against standard solution of sodium thiosulphate (hypo) solution using starch indicator. The number of bromine atoms consumed and time required is dependent upon the structure of amine. The reaction is generally completed in 10-45 minutes.
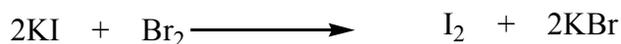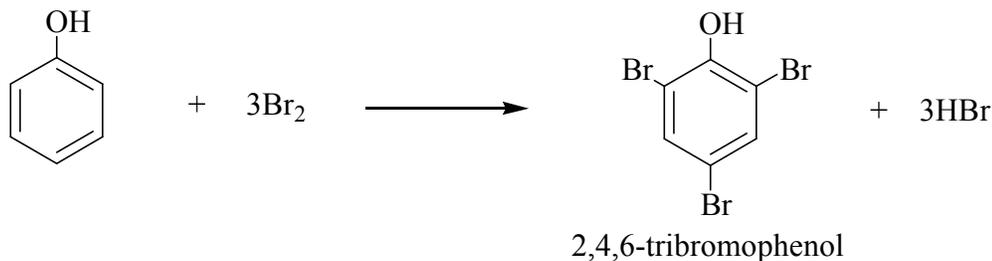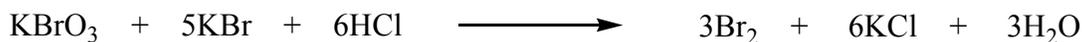
$$KBrO_3 \ + \ 5KBr \ + \ 6HCl \longrightarrow 3Br_2 \ + \ 6KCl \ + \ 3H_2O$$

2,4,6-tribromophenol

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

$$I_2 \ + \ Na_2S_2O_3 \longrightarrow Na_2S_4O_6 \ + \ 2NaI$$
(hypo)

Reagents:

1. Brominating mixture (~0.1N): In a 100 mL measuring flask dissolve 0.4 g of anhydrous $KBrO_3$ (potassium bromate) and 1.8 g of KBr (Potassium bromide) in distilled water and make up to 100mL.

2. Sodium thiosulphate solution (approximately 0.1N): Dissolve 6.2 g of sodium thiosulphate in distilled water and make up to 250 mL. For better result, this solution must be prepared one day before the experiment. Standardise the solution idometrically to know its exact normality using standard solution of potassium dichromate ($K_2Cr_2O_7$) or copper sulphate.

3. Potassium iodide solution (20%): Dissolve 15 g of potassium iodide in 75 mL of distilled water.

4. Indicator: 1% starch solution

5. Stock solution of aniline: Stock solution of phenol is generally prepared by dissolving 8 g of phenol in 1000 mL of water containing 1 to 2 mL of concentrated HCl. For the experiment 10 mL from the stock solution should be given to each student.

Procedure:

Make given solution (10 mL) of phenol up to 100 mL in a measuring flask. Pipate out 25 mL of this phenol sample in a 250 mL conical flask with stopper (iodine flask), to it add 25 mL of

bromate bromide solution along with 25 mL of distilled water. Add 5 mL of concentrated HCl to the reaction mixture, close the flask with stopper immediately, and shake well to mix the contents. Allow the flask to stand for 10 minutes with occasional shaking. Now add 25 mL of 20% potassium iodide solution to the reaction mixture and shake well. A dark yellow colour is developed due to the liberation of iodine and its dissolution in KI. Titrate the liberated iodine, which is equivalent to excess of bromine taken, with standard hypo (sodium thiosulphate) solution using starch as indicator. The end point is marked by the disappearance of blue colour. Perform a blank experiment without using aniline solution.

Calculation:

Volume of hypo used with the sample = V_1 mL

Volume of hypo used with the blank = V_2 mL

Normality of hypo solution = N/x

1000 mL of 1N hypo solution $\equiv$ 1 g equivalent weight of hypo $\equiv$ 1 g equivalent weight of Br

$\therefore$ (V_2-V_1) mL of N/x hypo solution $\equiv \left(\frac{V_2 - V_1}{1000}\right) \times \frac{N}{x}$ g equivalent weight of hypo or Br

$$= \left(\frac{V_2 - V_1}{1000}\right) \times \frac{N}{x} \times \frac{1}{6} \text{ moles of phenol}$$

Because from the reaction given above, 1 g mole of phenol = 6 g equivalent weight of Br

therefore 25 mL of the phenol sample conatins = $\left(\frac{V_2 - V_1}{1000}\right) \times \frac{N}{x} \times \frac{94}{6}$ g of phenol

(where 94 is the molecular weight of phenol)

Thus, 1 liter of phenol sample contains = $\left(\frac{V_2 - V_1}{1000}\right) \times \frac{N}{x} \times \frac{94}{6} \times \frac{1000}{25}$ g of phenol

$$= \left(\frac{V_2 - V_1}{150}\right) \times \frac{N}{x} \times 94 \text{ g of phenol}$$

Amount of phenol g/liter will be

$$= \left(\frac{V_2 - V_1}{150}\right) \times \frac{N}{x} \times 94$$

5.6 DETERMINATION OF IODINE VALUE OF AN OIL SAMPLE

Theory:

The iodine value or iodine number of an oil or fat is defined as the number of grams of iodine taken up by 100 g of oil and fat. Oils and fats are trimesters of glycerol with saturated and/or

unsaturated higher fatty acids. The degree of unsaturation of an oil or fat is measured in terms of iodine value. Linseed oil or cod liver oil etc. have a large proportion of unsaturated fatty acids, hence their iodine value is high, whereas olive oil or almond oil possesses a low iodine value. Iodine values of coconut, olive and linseed oil are 10, 88 and 108, respectively. Iodine value is determined by reacting a known volume of iodine monochloride solution in acetic acid (Wij solution) with the given oil or fat dissolved in $CHCl_3$ or CCl_4 and then titrating the unused iodine with standard sodium thiosulphate (hypo) solution using starch as indicator.

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

$$2KI \quad + \quad ICl \longrightarrow \quad I_2 \quad + \quad 2KCl$$

$$I_2 \quad + \quad \underset{(hypo)}{Na_2S_2O_3} \longrightarrow \quad Na_2S_4O_6 \quad + \quad 2NaI$$
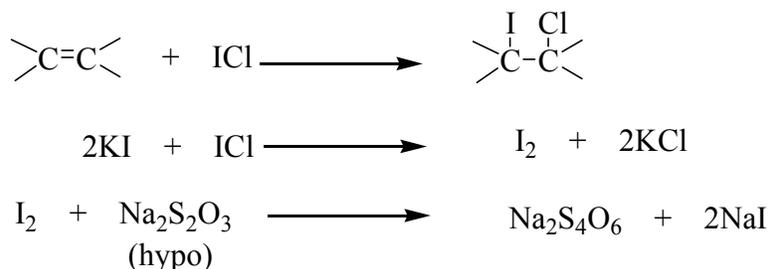
Reagents:

1. Wij's solution: This is a solution of iodine monochloride (ICl) in glacial acetic acid. Dissolve 3.0 g of iodine in 250 mL of glacial acetic acid and pass a slow current of chlorine gas through it until the deep red colour changes to orange yellow due to formation of iodine chloride. The Wij's solution must be stored in a well stoppered amber coloured bottle.

2. Sodium thiosulphate solution (approximately 0.1N): Dissolve 6.2 g of sodium thiosulphate in distilled water and make up to 250 mL. For better results, this solution must be prepared one day before of the experiment. Standardise the solution idometrically to know its exact normality using standard solution of potassium dichromate ($K_2Cr_2O_7$) or copper sulphate.

3. Potassium iodide solution (20%): Dissolve 15 g of potassium iodide in 75 mL of distilled water.

4. Indicator: 1% starch solution

Procedure:

Weigh accurately about 1.0 g of given oil or fat, dissolve it in 10 mL of chloroform or carbon tetrachloride in a 500 mL of conical flask. Pipate out 25 mL of Wij's solution and add to the flask containing solution of oil or fat. Mix the contents well and close the flask and keep in dark for 30 minutes with occasional shaking. Add 40 mL of 15% KI solution and 250 mL of water.

Shake the flask for 5-10 minutes and titrate the content against standard hypo solution using starch as indicator.

Standardization of the Wij's solution

Take 25 mL of the Wij's solution in a 500 mL conical flask and add 75 mL of 15% KI solution. Dilute the mixture with 250 mL of water and standardise the solution idometrically using 0.1 N standard copper sulphate or potassium dichromate solution and starch as indicator.

Calculation:

Weight of oil or fat = W g

Volume of hypo used with the sample = V_1 mL

Volume of hypo used for 25 mL of the Wij's solution blank = V_2 mL

Normality of hypo solution = N/x

1000 mL of 1N hypo solution ≡ 1 g equivalent weight of hypo ≡ 1 g equivalent weight of iodine

$\therefore$ $(V_2\text{-}V_1)$ mL of N/x hypo solution $\equiv \left(\frac{V_2 - V_1}{1000}\right) \times \frac{N}{x}$ g equivalent weight of iodine

$$= \left(\frac{V_2 - V_1}{1000}\right) \times \frac{N}{x} \times 127 \text{ moles of iodine}$$

(where 127 is the molecular weight of phenol)

▢ W g of the oil or fat takes up = $\left(\frac{V_2 - V_1}{1000}\right) \times \frac{N}{x} \times 127$ g of iodine

▢ 100 g of oil or fat will take up = $\left(\frac{V_2 - V_1}{1000}\right) \times \frac{N}{x} \times 127 \times \frac{100}{W}$ g iodine

▢ Iodine number of iodine value = $\left(\frac{V_2 - V_1}{10}\right) \times \frac{N}{x} \times \frac{127}{W}$

5.7 DETERMINATION OF SAPONIFICATION VALUE OF AN OIL SAMPLE

Theory:

The saponification value of an oil or fat is defined as the number of milligrams of KOH required to completely saponify 1 g of oil or fat. The magnitude of saponification value gives an idea about the average molecular weight of the oil or fat. The higher the molecular weight of the oil or fat, smaller is its saponification value. Soyabean oil and cotton seed oils have the saponification value in the range of 180-200. Coconut oil has saponification ~ 250. Butter fat having short chain fatty acids has saponification value in the range of 230-250.
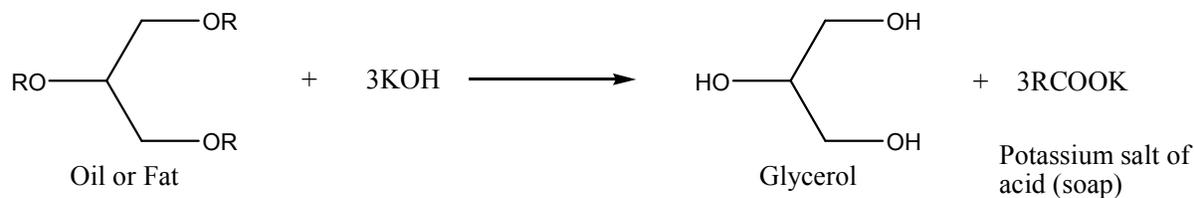
The saponification value of an oil or fat is determined by refluxing an accurately weighed amount of an oil or fat with an excess of alcoholic KOH solution and then titrating the unreacted KOH against a standard solution of an acid using phenolphthalein as indicator.

Reagents:

1. Standard 0.5N oxalic acid solution: Dissolve 15.75 g of oxalic acid in distilled water and make up to 500 mL.

2. 0.5N alcoholic KOH solution: Dissolve 6g of KOH pellets in 250 mL of 95% ethanol in stoppered bottle. Allow the solution to settle for about 4 hrs, filter and then standardise with 0.5N oxalic acid.

3. Indicator: Phenolphthalein.

Procedure:

Take 0.5 g of the oil or fat in a 250 mL conical flask fitted with an air condenser. Add 25 mL of 0.5N alcoholic KOH solution to the flask and reflux for 2 hours on a sand bath with frequent shaking. Cool the flask and add 4-5 drops of phenolphthalein indicator and titrate the remaining KOH with standard (0.5N) oxalic acid until the pink colour disappears. Perform a blank experiment without using the oil or fat.

Calculation:

Weight of oil or fat $= W$ g

Volume of 0.5N oxalic acid used with the sample $= V_1$ mL

Volume of 0.5N oxalic acid used for the blank $= V_2$ mL

Volume of 0.5N oxalic acid $(V_2\text{-}V_1)$ mL $\equiv (V_2\text{-}V_1)$ mL of 0.5N KOH solution

⮚   1000 mL of 1N KOH contains 56 g of KOH

∴ $(V_2\text{-}V_1)$ mL of *0.5N* KOH solution contains $\equiv 56 \times \left(\frac{V_2 - V_1}{1000 \times 2}\right)$ g of KOH

∴ Saponification value of the given oil or fat $= 56 \times \left(\frac{V_2 - V_1}{1000 \times 2}\right) \times \frac{1000}{W}$ mg of KOH

$$= 56 \times \left(\frac{V_2 - V_1}{W \times 2}\right) \text{ mg of KOH}$$

5.7 DETERMINATION OF DISSOLVED OXYGEN (DO) IN WATER SAMPLE

Theory:

Dissolved oxygen (DO) determination measures the amount of dissolved (or free) oxygen present in water or wastewater. It is also consumed by oxidation of organic matters or by reducing agents, etc. Aerobic bacteria and aquatic life such as fish need dissolved oxygen to survive. If the amount of free or DO present in the wastewater process is too low, the aerobic bacteria that normally treat the sewage will die. Dissolved oxygen in a water body is an index of physical, chemical and biological process going on in it. There are two main sources of dissolve oxygen in water:

1. Diffusion from air/absorption from air: It is a physical phenomenon and is influenced by temperature, pressure, movement of water and salinity.

2. Photosynthetic activity within water by vegetation. It is a biological phenomenon.

The optimum value for good water quality is 4-6 mg/liter of DO, which ensures healthy aquatic life in a water body. The determination of dissolved oxygen plays an important role in water-pollution control activities and water treatment process control. There are two methods for the determination of DO in water.

1. Electrode or oxygen meter method
2. Winkler's or iodometric method

1. **Electrode or oxygen meter method:** This method is quick, convenient and reasonably accurate.

Materials:

(i) Oxygen meter with dissolve oxygen probe

(ii)Electrical stirrer

(iii) 5% Sodium sulphite solution

Procedure:

Adjust the oxygen meter according to the operational manual. Dip the DO probe in 5% sodium sulphite solution with constant stirring. Set the meter to zero mark. Then dip the DO probe in constantly stirred water sample, and record the DO in mg/liter from the scale.

2. Winkler's Method:

On addition of mangnous sulphate to the water sample containing alkaline potassium iodide, mangous hydroxide is formed which is then oxidised by dissolve oxygen of water to basic manganic oxide. This on treatment with sulphuric acid liberates the iodine equivalent to that of dissolved oxygen originally present in the sample. The liberated iodine is treated with a standard sodium thiosulphate solution using starch as indicator.

$$MnSO_4 \ + \ 2KOH \longrightarrow Mn(OH)_2 \ + \ K_2SO_4$$

$$2Mn(OH)_2 \ + \ O_2 \text{ (dissolve oxygen)} \longrightarrow 2MnO(OH)_2$$
$$\text{(Basic mabganic oxide brown in colour)}$$

$$MnO(OH)_2 \ + \ 2H_2SO_4 \longrightarrow Mn(SO_4)_2 \ + \ 3H_2O$$
$$\text{Manganic sulphate}$$

$$Mn(SO_4)_2 \ + \ 2KI \longrightarrow MnSO_4 \ + \ K_2SO_4 \ + \ I_2$$

$$I_2 \ + \ Na_2S_2O_3 \longrightarrow Na_2S_4O_6 \ + \ 2NaI$$
$$\text{(hypo)}$$
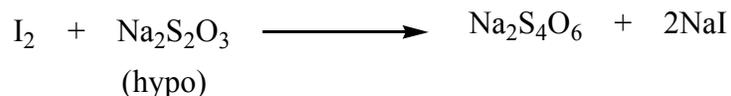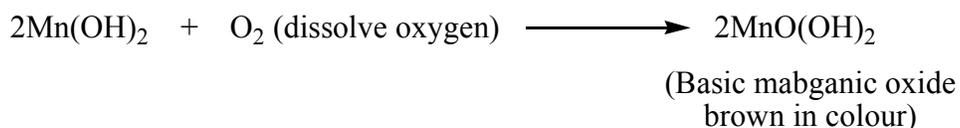
Reagents:

(i) **Mangnous sulphate solution:** Dissolve 100 g of mangnous sulphate in 200 mL of previously boiled distilled water and filter if necessary.

(ii) **Alkaline potassium iodide solution:** Dissolve 50 g of potassium iodide and 100 g of potassium hydroxide in 200 mL of previously boiled distilled water.

(iii) **Sodium thiosulphate solution (0.025N):** Dissolve 1.55 g of sodium thiosulphate in 250 mL of previously boiled distilled water and standardize the solution against standard potassium dichromate solution (0.025N) using starch indicator.

(iv) **Starch indicator:** 1% starch indicator

(v) **Concentrated sulphuric acid:** Specific gravity 1.84.

Procedure:

Take 100 mL of water sample in a 250 mL bottle with glass stopper or 250 mL BOD (biochemical oxygen demand) bottle. Add 2 mL of mangnous sulphate solution and 2 mL of alkaline potassium iodide solution to it. Shake the mixture well and allow the precipitate to settle and then add 2 mL of concentrated sulphuric acid to it. Shake the mixture well to dissolve the

precipitate. Titrate the liberated iodine against 0.025N solution of sodium thiosulphate using starch as indicator.

Calculation:

$$1000 \text{ mL of 1N } Na_2S_2O_3 \equiv 8 \text{ g oxygen}$$

$\therefore$ V mL of 0.025 N EDTA $= \dfrac{V \times 0.025 \times 8}{1000}$ g oxygen per 100 mL water sample

$$= V \times 0.025 \times 8 \text{ mg oxygen per 100 mL water sample}$$

$$= V \times 0.025 \times 8 \times 10 \text{ mg oxygen per 1000 mL water sample}$$

5.8 DETERMINATION OF BIOLOGICAL OXYGEN DEMAND (BOD) OF WATER SAMPLE

Theory:

The biochemical oxygen demand (BOD) may be defined as the amount of oxygen (O_2) required by microorganisms in the biochemical degradation or transformation of organic matter present in water. It is an indirect determination of the amount of biochemically degradable organic matter present in the water sample. It is the best available, single test for assessing organic pollution in a water body. The BOD is determined by the measurement of dissolved oxygen (DO) content of the sample before and after five days incubation at 20°C. Preliminary dilution (using dilution water) and aeration of the water sample is necessary to ensure that not all O_2 is consumed during incubation. Excess of dissolved oxygen must be present during the whole incubation. The water sample is seeded with microorganisms. The purpose of seeding is to introduce into the sample, a biological population capable of oxidizing the organic matter in the water sample. Where such microorganisms are already present, *i.e.*, in domestic waste water or surface water seeding is not necessary.

Reagents:

1. Phosphate buffer solution (pH 7.2): Dissolve 0.85 g of potassium hydrogen phosphate (KH_2PO_4), 2.175 g of dipotassium hydrogen phosphate (K_2HPO_4), 3.34 g of disodium potassium hydrogen phosphate heptahydrate ($Na_2HPO_4.7H_2O$) and 0.17 g og ammonium chloride (NH_4Cl) in 50 mL of distilled water and make up to a 100 mL.

2. Dilution water: Take required volume of distilled water in a suitable bottle and add 1 mL/liter of phosphate buffer of pH 7.2, 1 mL of $MgSO_4$ solution (22.5 g/liter), 1 mL of $CaCl_2$ solution (27.75 g/liter) and 1 mL of $FeCl_3$ (0.25 g/liter) solution.

3. Dilution of water sample: The dilution depends on the nature of the sample: 0.1-1% for industrial wastes, 1-5% for raw or settled sewage, 25-100% for polluted river water and 5-25% for oxidized effluents.

Procedure:

Aerate the water sample thoroughly by passing air into it for 5 minutes. Make a measured dilution of the sample with dilution water if BOD is greater than DO level. Seed with a little diluted domestic waste water (1.2 mL/liter). Measure DO on a suitable aliquot (D_1). Fill a screw-topped incubation bottle (250-300 mL) to the brim with the remaining diluted sample. Seal the bottle and incubate in the dark for 5 days at 20°C. Measures DO on aliquot of the sample (D_2).

Calculation:

$$\text{mg/liter BOD} = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$$

where; D_1 = Dissolved oxygen of diluted sample

D_2 = Dissolved oxygen of diluted sample after incubation

B_1 and B_2 are the DO of dilution water containing seed before and after incubation, respectively, f = ratio of seed in sample to seed in control, and P = decimal fraction of the sample water used.

5.9 DETERMINATION OF CHEMICAL OXYGEN DEMAND (COD) OF WATER SAMPLE

Theory:

The chemical oxygen demand (COD) can be defined as the amount of oxygen (mg/liter) required for the oxidation of organic matter present in water. This is an important parameter for assessing water quality and is more scientific than traditional concept of BOD. In COD test, water sample is subjected to a chemical oxidation which oxidises both biologically oxidisable and biologically inert organic matter. Thus the COD value is always higher than the BOD value. Although, COD determination is a very quick experimental process in comparison to BOD determination (5 days), it does not differentiate between biodegradable and non-biodegradable materials. The

determination of COD of water sample is based on the chemical oxidation of the present organic matters with potassium dichromate and sulphuric acid. The water sample is refluxed with a known amount of potassium dichromate in 50% of sulphuric acid and the excess of dichromate is titrated against standard ferrous ammonium sulphate solution. The amount of dichromate thus consumed is proportional to the amount of oxygen required to oxidise the organic matter present in the water sample.

$$6FeSO_4(NH_4)_2SO_4.6H_2O \ + \ K_2Cr_2O_7 \ + \ 7H_2SO_4 \longrightarrow \ K_2SO_4 \ + \ Cr_2(SO_4)_3 \ + \ 3Fe_2(SO_4)_3 \ +$$
$$6(NH_4)_2SO_4 \ + \ 43H_2O$$
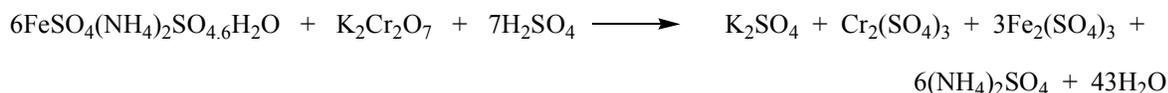
Reagents:

1. Potassium dichromate solution (0.25N): Dissolve 3.0648 g of potassium dichromate in 250 mL of distilled water.

2. Powdered silver sulphate: It is used as an oxidising catalyst for straight chain aliphatic compounds, aromatic hydrocarbons and pyridine.

3. Powdered mercuric sulphate: It traps the chloride ions present in water sample and prevents their interference.

4. Concentrated sulphuric acid: Specific gravity 1.84

5. Phenanthroline ferrous sulphate (ferroin) indicator solution: Dissolve 1.48 g of 1-10 (ortho) phenanthroline monohydrate, together with 0.70 g of $FeSO_4.7H_2O$ in 100 mL of distilled water. This indicator may be purchased already prepared.

6. Standard ferrous ammonium sulphate (0.25 N): Dissolve 98.0 g of $Fe(NH_4)_2(SO_4)_26H_2O$ in distilled water. Add 20 mL of conc. H_2SO_4, cool and dilute to 1 liter. This solution must be standardized daily against standard 0.25 N $K_2Cr_2O_7$ solution using 2-5 drops of the ferroin indicator. The end point is indicated by the colour changes from blue green to reddish brown.

Procedure:

Take 20 mL of water sample in 250 mL of round bottom flask, add a pinch of silver sulphate powder and mercuric sulphate powder, then 10 mL of 0.25N potassium dichromate solution followed by so mLof concentrated sulphuric acid. Add few pieces of porcelain to the flask to checking the bumping, fit a water condenser and reflux the mixture for at least 2 hours. Now cool the flask and transfer the content in a 500 mL of conical flask and dilute it with 150 mL of

distilled water. Add 2-3 drops of ferroin indicator and titrate is against 0.25N ferrous ammonium sulphate solution. The end point is the sharp colour change from blue green to reddish brown. Conduct a blank experiment using distilled water in place of the water sample. The amount of other reagents added are the same as that of added for the sample.

Calculation:

$$COD\ mg/liter = \left(\frac{(V_2 - V_1) \times N \times 1000 \times 8}{Volume\ of\ sample\ in\ mL} \right)$$

V_1 = Volume of ferrous ammonium sulphate solution used against the sample

V_2 = Volume of ferrous ammonium sulphate solution used against the blank

N = Normality of ferrous ammonium sulphate

The Equivalent weight of oxygen = 8

5.11 Spectroscopic estimations

5.11.1 Spectroscopic estimation of Amino acids

Theory:

Amino acids are known as the building blocks of all proteins. There are 20 different amino acids commonly found in proteins. Amino acids are comprised of a carboxyl group and an amino group attached to the same carbon atom (the α carbon). They vary in size, structure, electric charge and solubility in water because of the variation in their side chains. Detection, quantification and identification of amino acids in any sample constitute important steps in the study of proteins.

Most amino acids, when treated with ninhydrin, are oxidatively determined. The resulting ammonia react with ninhydrin and its reduced product to give a blue substance diketo hydrindyindene diketo hydrindiamimine, which has an absorption maxima at 570 nm. The reaction takes place between pH 4 to 8.

ninhydrin

purple colored product
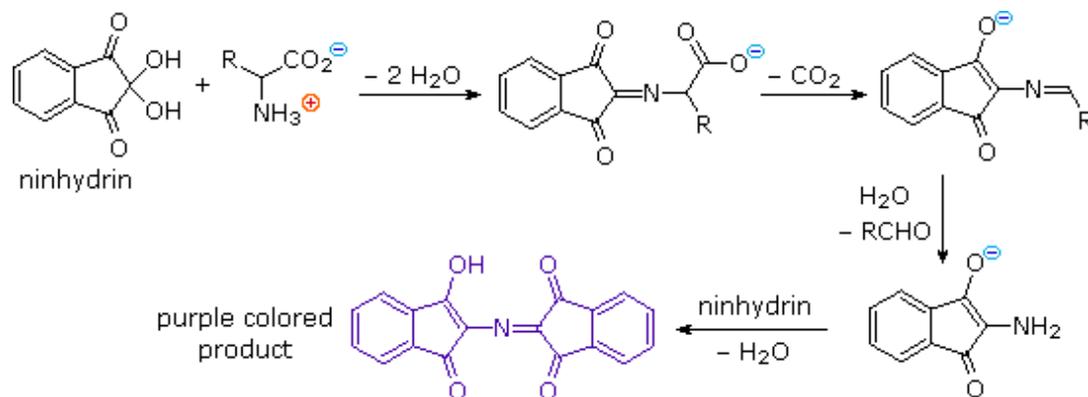
Reagents:

1. *4N* sodium acetate buffer, pH 5.5: 540 g of sodium acetate is dissolve in 400 mL of water with heating if necessary, cool the solution and add 125 mL of glacial acetic acid, make up the volume up to 1 liter by adding distilled water. The pH of the resulting solution is 5.5. The buffer can be stored at 4°C.

2. Ninhydrin reagent: 2 g of ninhydrin and 300 mg of hydrindantin is dissolved in 75 mL of ethylene glycol monomethyl ether. Stir the resulting solution very gently so that no air bubbles are formed. 25 mL of the buffer having pH 5.5 is then added and resulting reddish reagent solution is transferred immediately to a brown coloured bottle.

3. Ethanol (50 percent V/V)

4. Amino acid: Dissolve 25 mg of amino acid in 0.1 N HCl (50 mL); the concentration of this stock solution is 500 mg/L.

Procedure:

Pipette out aliquots of the amino acid stock solution (0 mL, 0.5 mL, 1.5 mL, 2 mL and 2.5 mL) into a series of test tubes. Make the volume in each test tube to 5 mL by adding requisite amount of water. Also take 4 mL of solution of amino acid of unknown concentration [note the concentration of such unknown solution is not more than 500 mg/L; this should be between 100 mg/L to 500 mg/L]. The ninhydrin reagent (2 mL) is then added to each test tube; mixed well and both tubes are kept in boiling water for 15 minutes. On heating the content purple colour is obtained.

Observations Table:

Test Tube	mL of amino acid (V₁)	Distilled water added (V₂)	Ninhydrin reagent added	Concentration of amino acid in mg/L $C_2 = \dfrac{C_1 V_1}{V_2}$	Absorbance
1 (blank solution)	0	5	2	0	
2	0.5	4.5	2	50	
3	1.0	4.0	2	100	
4	1.5	3.5	2	150	
5	2.0	3.0	2	200	
6	2.5	2.5	2	250	
7 (unknown solution)	5.0	0	2	?	

C_1 = concentration of stock solution in mg/L (500 mg/L)

Set the wavelength on the spectrophotometer to 570 nm. Place a blank (3 mL) in a cuvette and insert this cuvette in the reference holder and zero the instrument. Add 3 mL of the solution from test tube-1 to the second cuvette, wipe the transparent sides and insert it in to the sample holder of spectrophotometer. Record the absorbance. Repeat this step for rest of the solutions (i.e. 2-7) using the same cuvette and record the absorbance. Remember to rinse the cuvette before the next run.

Draw a standard graph between absorbance versus amino acid concentration. The concentration of the amino acid is extrapolated from the standard graph.

5.11.2 Spectroscopic estimation of carbohydrate

Carbohydrates (generally hexose) form furfural in presence of conc. H_2SO_4 which condense with 9,10-dihydro-9-oxo-anthracene (anthrone) to yield a blue green coloured complex. The absorption maximum of this coloured compound is generally observed at 620 nm.

Procedure:

(i) Anthrone reagent (2% in conc. H_2SO_4): Dissolve 200 mg anthrone in 100 mL of cold concentrated H_2SO_4 and store it at 4°C.

(ii) Standard glucose solution (10 mg in 100 mL of distilled water).

Pipette out aliquots of the standard glucose solution (0 mL, 1.0 mL, 1.5 mL, 2 mL and 2.5 mL) in to a series of test tubes. Make the volume in each test tube to 5 mL by adding requisite amount of water. Take 5 mL of unknown glucose solution in the sixth test tube. Add 4 mL of anthrone reagent to each test tube and mix rapidly and carefully. Place the test tubes in boiling water bath for 10 minutes with a marble on top to prevent the loss of water, and then cool the contents.

Observations Table:

Test Tube	mL of glucose (V_1)	Distilled water added (V_2)	anthrone reagent added	Concentration of amino acid in mg/L $C_2 = \dfrac{C_1 V_1}{V_2}$	Absorbance
1 (blank solution)	0	5	4	0	
2	1.0	4.0	4	20	
3	1.5	3.5	4	30	
4	2.0	3.0	4	40	
5	2.5	2.5	4	50	
6	3.0	2.0	4	60	
7 (unknown solution)	5.0	0	4	?	

C_1 = concentration of stock solution in mg/L (100 mg/L)

Set the wavelength on the spectrophotometer to 620 nm. Place a blank (3 mL) in a cuvette and insert this cuvette in the reference holder and zero the instrument. Add 3 mL of the solution from the test tube-1 to the second cuvette, wipe the transparent sides and insert it in to the sample holder of spectrophotometer. Record the absorbance. Repeat this step for rest of the solutions (i.e. 2-7) using the same cuvette and record the absorbance. Remember to rinse the cuvette before the next run.

Draw a standard graph between absorbance versus amino acid concentration. The concentration of the amino acid is extrapolated from the standard graph.

5.11.3 Spectroscopic estimation of protein

Principle: The –CONH– group in the protein molecule react with copper sulphate in alkaline medium to give purple coloured complex. The absorption maximum of this complex is 540 nm.

Since all protein contains the –CONH– group, the method is quite specific and there is no interference from other compounds. Some substances like urea and biuret interfere because they posses –CONH– groups. Other interfering materials are reducing sugars like glucose which also react with copper sulphate.

Reagents:

1. Biuret reagent: Dissolve 4.25 g of sodium potassium tartrate, 1.5 g of copper sulphate and 2.5 g of KI in 500 mL of water. Add 4 g of NaOH in the above solution and dissolve it. Make the volume of the solution to one liter.

2. Standard solution of protein: Dissolve 100 mg of protein in 50 mL of water or 0.1 M sodium acetate acetic acid buffer pH 5.5 containing 0.2 g NaCl. Bovin serum albumin is commonly used as standard.

3. Protein solution of unknown concentration: Prepare protein solution of unknown concentration by dissolving 50 to 80 mg of protein in 50 mL of water. This solution is provided to the students.

Procedure:

Pipette out aliquots of the standard protein solution (0 mL, 1.0 mL, 1.5 mL, 2 mL and 2.5 mL) into a series of test tubes. Make the volume in each test tube to 5 mL by adding requisite amount of water. Take 5 mL of unknown protein solution in the sixth test tube. Add biuret reagent (6 mL) to each conical flask and mix well. Keep the flask at 37°C for 10 minutes during which a purple colour will develop. The Absorbance of each flask is measured at 540 nm. Prepare a standard curve between protein concentration v/s absorbance. Draw the standard graph. Determine the concentration of unknown protein solution from this standard graph.

Observations Table:

Test Tube	mL of protein (V₁)	Distilled water added (V₂)	biuret reagent added	Concentration of amino acid in mg/L $C_2 = \dfrac{C_1 V_1}{V_2}$	Absorbance
1 (blank	0	5	6	0	

solution)					
2	1.0	4.0	6	400	
3	1.5	3.5	6	600	
4	2.0	3.0	6	800	
5	2.5	2.5	6	1000	
7 (unknown solution)	5.0	0	6	?	

C_1 = concentration of stock solution in mg/L

5.11.4 Spectroscopic estimation of Ascorbic acid

Principle: Ascorbic acid is measured by dinitrophenylhydrazine method. This procedure is based on the oxidation of ascorbic acid to dehydroasorbic acid followed by coupling with 2,4-dinitrophenylhydrazine under controlled conditions to give red coloured osazone. A comparison of colour produced in the sample and ascorbic acid standard solution is used as a means of determining ascorbic acid content.

Reagents:

1. 2,4-dinitrophenylhydrazine: 2 g of 2,4-dinitrophenylhydrazine is dissolved in 100 mL 9 N H_2SO_4 and filtered.

2. 6% tricholoroacetic acid (TCA)

3. Acid washed norit: 200 g of norit is suspended in 1 L of 10% HCl, heated to boiling and filtered under suction. The cake is removed and stirred with 1 L of 10% HCl and refiltered. The process is repeated until the washing gives a negative test for ferric ions. The norit is then dried overnight at 110-120°C **.**

4. 85% H_2SO_4: It is prepared by adding 85 mL of conc. H_2SO_4 in to 100 mL of water.

5. Thiourea solution: 10 g of Thiourea dissolved in 100 mL of 50% (v/v) aqueous alcohol.

6. Stock solution of ascorbic acid: Dissolve 20 mg of ascorbic acid in 100 mL of 6% TCA.

Procedure: Pipette out 0, 1, 3, 6, 8 and 10 mL of stock solution in a series of six conical flasks, and make the volume of each flask to 100 mL by 6% TCA. Add 300 mg of acid washed norit in

each flask with vigorous shaking. Filter each solution if solution is not clear. These solutions are called norit filtrates. Norit oxidises ascorbic acid to dihydroascorbic acid in the presence of TCA. Mark one of the solutions (say solution 3) as unknown. Solution 1 will give blank. Take six test tubes and pipette out 1.6 mL of norit filtrate from each flask and transfer it into each test tube. Add one drop of Thiourea solution and 0.4 mL of 2,4-dinitrophenylhydrazine reagent in all six test tubes. These tubes are incubated at 37°C for three hrs and then placed in an ice bath.

Now add 2 mL 85% H_2SO_4 drop wise with stirring in each test tube. Colour is developed in each test tubes except tube no. 1. Test tube no 1 contains blank (no ascorbic acid solution). The test tubes are removed from the ice water bath and allowed to stand for 30 minutes at room temperature. Determine the absorbance of each solution at 540 nm setting the blank at zero absorbance. Prepare a standard curve between absorbance and concentration and determine the concentration of unknown from it. The concentration of stock solution is 20 mg in 100 mL (0.2mg/L).

Observations Table:

1	2	3	4	5	6	7	8
Vol of stock solution (V_1) (mL)	Vol of stock solution + 6% TCA (V_2) (mL)	Conc. of ascorbic acid (mg/100mL)	Amount of norit added in solution of col 2	Volume of norit filtrate (mL)	Vol of 2,4-dinitrophenylhydrazine added to solution of col. 5	Vol of 85% H_2SO_4 added in solution of col 5	Absorbance
Blank (0 mL)	100	0	300 mg	1.6	0.4 mL	2 mL	
1	100	0.2	300 mg	1.6	0.4 mL	2 mL	
3	100	0.6	300 mg	1.6	0.4 mL	2 mL	
6	100	1.2	300 mg	1.6	0.4 mL	2 mL	
8	100	1.6	300 mg	1.6	0.4 mL	2 mL	
unknown	100	?	300 mg	1.6	0.4 mL	2 mL	

5.11.5 Spectroscopic estimation of Aspirin in APC tablets

Principle: APC tablets are a mixture of aspirin, phenacetin and caffeine. Aspirin has absorption maximum at 227 nm. A powdered tablet is dissolved in CH_2Cl_2 and the aspirin is separated from the phenacetin and caffeine by extracting it into aqueous sodium bicarbonate solution. The separated aspirin is black-extracted in to CH_2Cl_2 by acidifying the aqueous layer and is then measured spectrophotometrically at 277 nm.

Reagents and Chemicals:

1. Dichloromethane (CH_2Cl_2)

2. 4%wt/vol $NaHCO_3$ solution (chilled)

3. 1M H_2SO_4

4. Standard solution: Prepare 100 mg/L, 40mg/L, 20mg/L and 10 mg/L standard solutions of aspirin in CH_2Cl_2 as follows:

Take 25 mg of aspirin and transfer to 100 mL volumetric flask. Dissolve the aspirin in CH_2Cl_2 and dilute it to the mark with CH_2Cl_2. Take 1mL, 2mL, 4mL and 10mL from stock solution in four different volumetric flask of 25 mL capacity. Dilute them up to mark with CH_2Cl_2 to prepare the 10, 20, 40 and 100mg/L solutions, respectively. Mark these solutions as 1, 2, 3 and 4 respectively.

Procedure:

Take the weight of one tablet and record it. This should be equivalent to about 220 mg of aspirin, 160 mg of phenacetin and 30 mg of caffeine. Cut the tablet into quarters and take its weight. Crush it to fine powder in a beaker. Add 20 ml of CH_2Cl_2 under constant stirring and transfer the mixture to a 100 mL separatory funnel, rinse the beaker with little more CH_2Cl_2. Extract the aspirin from the CH_2Cl_2 solution with two 10 mL portion of 4% $NaHCO_3$, add two drops of conc. HCl and then extract with 5 mL of water. Wash the combined aqueous extracts with three 10 mL portions of CH_2Cl_2. Leave the aqueous extract in the separatory funnel.

Acidify the bicarbonate solution (aqueous extract which is still in the separatory funnel), with 6 mL of 1N H_2SO_4. This step should be performed without delay, to avoid hydrolysis of the aspirin. The acid must be added slowly in small portions. Mix well only after most of the CO_2 evolution has ceased. The pH at this stage should be 1 to 2. Extract the acidified solution with

eight separate 10 mL portions of CH_2Cl_2 and filter through a CH_2Cl_2-wet filter paper into a 100 mL volumetric flask. Dilute the solution to 100 mL. Dilute further a 5 mL portion of this solution to 25 mL with CH_2Cl_2 in a 25 mL of volumetric flask. This is the solution of unknown concentration. Mark this solution as solution 5.

Record the absorbance versus wavelength curve for the standard solution (any one of the solutions, i.e. solution 1 to 4). The absorbance maximum of this solution will be 277. Determine the absorbance of solutions 1, 2, 3 and 4 at 277 nm. Prepare the standard curve between the absorbance and concentration and determine the concentration of unknown solution from it.

5.11.6 Spectroscopic estimation of Cholesterol from blood sample

Principle:

Acetic anhydride reacts with cholesterol in chloroform solution to produce a characteristic blue green colour. The exact nature of chromophore is not known but the reaction probably includes acetylation of the hydroxyl group at position-3 as well as some rearrangement reaction in the molecule. This method of estimation is known as Libermann-Burchard method. This method involves:

- Treatment of blood or serum with alcoholic KOH to liberate the cholesterol from lipoprotein and to saponify the cholesterol esters. This gives cholesterol having hydroxyl group at position-3.
- Extraction of cholesterol into a known volume of petroleum ether after dilution of alcoholic solution with water and
- Measurement of cholesterol in an aliquot of petroleum ether extract by means of Libermann-Burchard reagent.

Reagents:

1. 33% KOH (w/v): 10 g of KOH pallets dissolved in 20 mL of water.

2. Alcoholic KOH: 6 mL of 33% KOH is made up to 100 mL with absolute alcohol. This solution is prepared fresh before use.

3. Standard cholesterol (40 mg/100 mL): 100 mg of cholesterol is dissolved in 250 mL of petroleum ether.

4. Modified Libermann-Burchard reagent: 20 mL of acetic anhydride is chilled to a temperature lower than 10°C in glass stoppered flask and 1 mL of concentrated H_2SO_4 is added.

The well shaken mixture is kept for 10 minutes at 10°C and then added to 10 mL 0f glacial acetic acid in it. The mixture is allowed to warm to attain the room temperature. This reagent is used within 1 hr.

Procedure:

Blood or serum 0.5 mL is taken into a 25 mL glass stoppered test tube to which 5 mL of freshly prepared alcoholic KOH solution is added. The well shaken test tube is then incubated in a water bath at 40°C for 1 hr. After cooling the contents at room temperature, 10 mL of petroleum ether is added to it. The mixture is then transferred in a separating funnel. 5 mL of water is then added to it and whole mixture is vigorously shaken for about one minute. Allow the separation of two layers. Remove the petroleum ether extract from the funnel to test tube or conical flask. Evaporate the petroleum ether to dryness on a boiling water bath. Cool and dissolve the residue in 2 mL of chloroform. This will be the unknown solution whose concentration is to be determined.

Pipette out 0, 1, 2, 3 and 4 mL of standard cholesterol solution in a series of test tubes(mark them as 1,2,3 4 and 5 respectively). Evaporate petroleum ether of all the test tubes to dryness on a boiling water bath. Add 2 mL chloroform in each test tube and dissolve the residue in it. Add 6 mL of Libermann-Burchard reagent in the test tube 1 first and then at regular intervals of 1 minute to the test tubes 2, 3, 4 and 5. Finally add the 6 mL of Libermann-Burchard reagent to test tube containing unknown solution. Determine the absorbance of each solution after 30 minutes after the addition of the reagent at 620 nm setting the blank to zero absorbance.

Prepare a standard curve between absorbance and concentration and determine the concentration of unknown solution by extrapolation of the curve.

5.11 SUMMARY

- The present unit comprises the different estimation methods of hydroxyl group, amines and phenols.
- Estimate the percentage and number of hydroxyl group present in a given organic compound
- Estimate the percentage and number of amine present in a given organic compound using different methods
- Estimate the percentage and number of phenol present in given organic compound
- Determine the iodine and saponification values of oils and fats.

- Determine the dissolved oxygen, biological oxygen demand and chemical oxygen demand in water sample.

- Spectroscopic estimation of various synthetic and natural organic compounds is also discussed in the present unit.