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Experiment 1: Estimation of Ni by gravimetry and complexometric titration.

Aim: Determine Gravimetrically and complexometrically the amount of Ni in the given solution.

Requirements: 1.0 % alc. solution of DMG, given nickel sample, solid EDTA,

Theory: Analysis of nickel is important especially in minerals and alloys. Nickel can be determined exactly by gravimetric method, where it is precipitated as dimethylglyoxime complex. However, the gravimetric methods, although very precise, are also time consuming. If one does not need great accuracy but prefers to shorten the time of analysis, an exploit the inclination of Ni to form coordination compounds, also with EDTA.

Nickel (II) forms a red colored precipitate with an alcoholic solution of dimethylglyoxime, $H_2C_4H_6O_2N_2$, in a slightly alkaline medium.
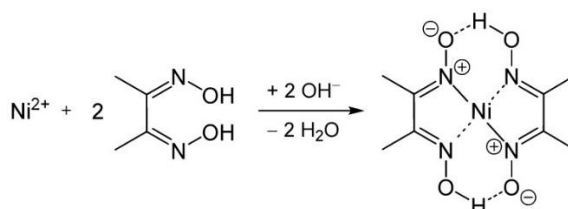
Determination of nickel using EDTA titrant is carried in alkaline environment. It is better if the environment is slightly alkaline at the beginning of reaction, and becomes more alkaline (from ammonia added). Indicator is murexide, changing color from yellow to violet. Complexing of nickel(II) by EDTA goes rather slowly, i.e. titration should be performed not too fast.

Reaction taking place during titration is

$$Ni^{2+} + EDTA^{4-} \rightarrow NiEDTA^{2-}$$
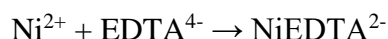
Procedure:

Part I : Gravimetry

1. Pipette out 20 cm^3 of given solution into 400 cm^3 beaker.
2. Add 100.0 cm^3 distilled water using a graduated cylinder.
3. Add concentrated NH$_3$ (1-2 mL) until a faint odor of NH$_3$ can be detected.
4. Heat the solution to 60° to 80 ^{o}C (do not boil but feel hot from outside of the beaker,) and then, add 20.0 cm^3 of 1% alcoholic solution of DMG.

5. Add immediately ammonia solution dropwise directly to the solution till the precipitation is complete and the solution smells of ammonia. Then add little excess of ammonia.

6. Allow the beaker to stand on water bath for 20-30 min.

7. Filter the cold solution through a sintered glass crucible.

8. Wash the ppt with cold water several times till free from chloride ions.

9. Dry the crucible at 110-120 degrees for 45 min in hot air oven.

10. Cool in a desiccator and weigh until constant weight is obtained.

Part II: Volumetric analysis

1. Prepare 250 cm^3 of 0.02 M EDTA solution.

2. Pipette out 10 cm^3 of Nickel solution into 150 cm^3 conical flask.

3. Add about 25cm^3 of distilled water.

4. Add 5 cm^3 of 10% ammonium chloride and ammonia (ammonia-ammonium chloride buffer) to obtain pH around 8.

5. Add a pinch of murexide indicator.

6. Titrate slowly with 0.02M EDTA solution till the solution color changes from yellow to violet.

Observations:

Part I: Gravimetry

    1. Weight of empty G3 crucible = W_1 = _____g

    2. Weight of crucible + precipitate = W_2 = _____g

    3. Weight of the residue = $W_2 - W_1$ = C = _____g

Part II: Volumetric analysis

   Principle:

   Burette:

   Conical Flask:

   Indicator:

End point:

Burette Reading	Pilot (cm³)	I (cm³)	II (cm³)	III (cm³)
Initial				
Final				
Difference				

C.B.R. = _____ cm³

Calculations:

Part I: Gravimetry

Mass of Ni(DMG)^2 precipitate = C g = …….. g

We know that one mole of Ni (DMG)$_2$ contain one mole of Ni^{2+} ions.

Thus, 288.7 g (1 mole) of Ni (DMG)$_2$ contains 58.7 g (1 mole) of Ni^{2+} ions.

1 g of Ni (DMG)$_2$ would contain = 58.7 / 288.7 g of Ni^{2+} ions

$\therefore$ C g of Ni (DMG)$_2$ would contain = A g = C $\times \dfrac{58.7}{288.7}$ g of Ni^{2+} ions

20 cm³ of the diluted solution contains A g = _____ g of Ni^{2+} ions

$\therefore$ 100 cm³ of the diluted solution contains $5A$ g = _____ g of Ni^{2+} ions

Part II: Volumetric analysis

10 cm³ of the diluted solution required x cm³ of 0.02 M EDTA

$\therefore$ 100 cm³ of the diluted solution required $10x$ cm³ of 0.02 M EDTA

1000 cm³ of 1M EDTA = 58.7 g of Ni

$\therefore$ $10x$ cm³ of 0.02 M EDTA = $\dfrac{58.7 \times 10x \times 0.02}{1000}$ g of Ni

= _____ g of Ni

Results: Amount of Ni (II) present in the given solution = Gravimetrically = _____ g

Volumetrically = _____ g

Experiment 2: To determine the energy of activation of the acid catalysed hydrolysis reaction of methyl acetate.

Aim: To determine the energy of activation of the acid catalysed hydrolysis reaction of methyl acetate.

Theory: This hydrolysis is a very slow reaction. During its kinetic study it is catalysed by a mineral acid. The reaction is represented as:

$$CH_3COOCH_3 + H_2O \xrightarrow{H^+} CH_3COOH + CH_3OH$$
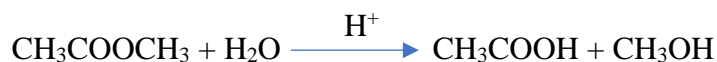
Specific reaction rate is related to energy of activation by the Arrenhius equation –

$$k = Ae^{-E_a/RT}$$

$$\log k = \log A - \frac{E_a}{2.303\ RT}$$

k – specific reaction rate

A – constant

E_a – Energy of activation

R – Gas constant

T – Temperature in Kelvin

A hydrolysis reaction is studies kinetically at different temperatures and k value at each temperature is determined. A graph of log k is plotted against 1/T, thus E_a can be calculated from the slope of this graph.

Requirements: 0.5 N HCl solution, 0.1 N NaOH solution, methyl acetate, phenolphthalein indicator, glass stoppered bottle, 5 and 25 cm^3 pipettes, conical flask, burette etc.

SET-I (RT) - Pipette out 5 cm^3 of methyl acetate in a reagent bottle, cork and keep in a water bath. Pipette out 100 cm^3 of 0.5N HCl in another bottle, cork and keep in the water bath. Note RT. At a noted time add the acid to the ester. Shake. After 5 min. pipette out 5cm^3 of the reaction mixture in a conical flask containing ice and phenolphthalein. Titrate against 0.1N NaOH . End point is light pink colour. Repeat at 10, 15, 20, 25, 30 min.

<u>SET-II</u> (10ºC above RT)-Pipette out 20cm^3 of methyl acetate in a reagent bottle, cork and keep in the thermostat. Pipette out 100 cm^3 of 0.5N HCl in another bottle, cork and keep in the thermostat. At a noted time add 5 cm^3 of the ester to the acid. Shake. After 5 min. pipette out 5cm^3 of the reaction mixture in a conical flask containing ice and phenolphthalein. Titrate against 0.1N NaOH. End point is light pink colour. Repeat at 10, 15, 20, 25, 30 min.

Plot a graph of log (a – x) vs. t min for both sets. Calculate K_1, K_2 and E_a.

Observations:

       T_∞ = _____ cm^3 (for both sets)

SET- I: T_1 =   + 273 = _____K

Time t (min)	Titre Reading T_t cm^3	a- x= T_∞ - T_t	log(a- x)
5			
10			
15			
20			
25			
30			

SET-II: T_2 =   + 273 = _____K

Time t (min)	Titre Reading T_t cm^3	a- x= T_∞ - T_t	log(a- x)

Graph:

SET - I

$K_1 = -(\text{slope} \times 2.303)$

$\log(a - x)$

t min.

SET - II

$\log(a - x))$

$K_2 = -(\text{slope} \times 2.303)$

t min.

Calculations:

(1) $\dfrac{K_2}{K_1} =$

(2) $E = \dfrac{2.303 \times 8.314 \times T_1 \times T_2}{(T_2 - T_1)} \cdot \log \dfrac{K_2}{K_1}$

Results:

(1) $T_1 =$ _____ K $K_1 =$ _____ min^{-1}

(2) $T_2 =$ _____ K $K_2 =$ _____ min^{-1}

(3) Energy of activation $E =$ _____ J

Experiment 3: To determine the strength of weak acid by titrating against strong base conductometrically.

Aim: To determine the strength of weak acid by titrating against strong base conductometrically.

Given: Solution of weak acid (Acetic acid) and 0.1N NaOH. Conductivity cell.

Procedure:

1. Dilute the given solution to 100 cm^3 in the volumetric flask using distilled water.

2. Standardize the instrument using the built in standard conductance.

3. Pipette out 10 cm3 of this diluted solution into a beaker and add sufficient quantity of water till the conductivity cell electrodes are properly immersed in the solution. Adjust the burette on the beaker which is filled with 0.1N NaOH.

4. Stir the solution carefully and record its conductance reading from conductometer.

5. Add 0.5 cm^3 of 0.1N NaOH from the burette and stir the solution again. Note the new conductance reading in the observation table.

6. Repeat the step 5 till the values of the conductance that have been successively decreasing, start to increase.

7. Take 7 to 8 more readings after this stage.

Observation:

Volume of alkali from burette V (cm^3)	Conductance **S**

Graph: Plot a graph of Conductance vs Vol. of alkali added
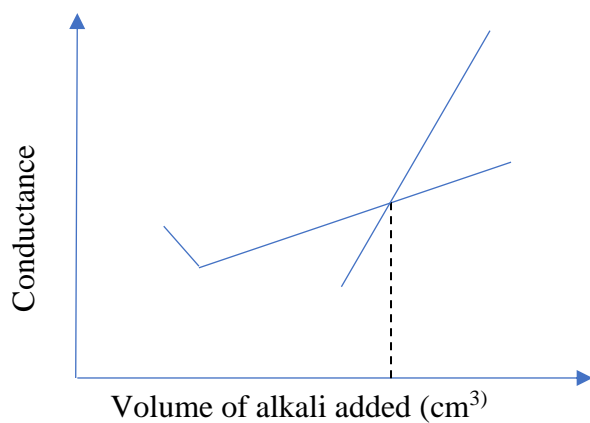
Calculations:

Volume of 0.1N NaOH required for the equivalence point = A cm^3 =_____ cm^3

CH3COOH   NaOH

N_1V_1 = N_2V_2

$$N_1 \; = \; \frac{N_2V_2}{V_1} \; =$$

Strength of acetic acid = X_1 = Normality × Eq. Wt. = $N_1 \times 60$ gdm^{-3}

Amount of acetic acid = $(X_1 \times 100)/1000$ g

Results:

1. Volume of 0.1N NaOH required for end point = A =_____

2. Normality of the given acid = N_1 = _____

3. Strength of the given acid = X_1=

4. Amount of acid present in the given solution = _____g.

Experiment 4: To determine the amount of Copper ions in the given solution by using spectrophotometer

Aim: To determine the λ_{max} of Cu-NH$_3$ complex and estimate the amount of Copper ions in the given solution by using Calibration Curve method and calculate percentage error.

Requirements: 100 cm^3 standard measuring flask, 10 cm^3 graduated pipette, burette, Spectrophotometer, 1000ppm solution of CuSO$_4$, distilled water.

Procedure:

1. Prepare 1000 ppm of copper by dissolving 0.393 g of copper sulphate pentahydrate in 100cm^3 standard measuring flask.
2. Pipette out 10cm^3 of 1000 ppm solution into 100 cm^3 standard flask and dilute it upto the mark with distilled water to get 100 ppm solution.
3. From the stock solution pipette out 0,1,2,3,4,5 and 6 cm^3 into seven serially numbered standard measuring flask.
4. To each flask add 5cm^3 of ammonia solution and immediately cork the flask.
5. Dilute each solution upto the mark using distilled water.
6. Dilute the given unknown solution upto the mark with distilled water. Pipette out 10 cm^3 of this solution into another 100 cm^3 flask. Repeat steps 4 and 5.
7. Take 6 ppm solution and find out the maximum absorbance by taking reading at different wavelengths ranging from 400-800 nm.
8. Note down the wavelength at which the solution gives maximum absorbance. This is λ_{max}.
9. Record the absorbance of all the other solutions at λ_{max} in increasing concentration.
10. Plot a graph of absorbance against concentration.

Observations:

Selection of λ_{max}

Sr. No.	Wavelength	Absorbance

Sr. No.	100 ppm Cu(II) solution (cm^3)	Ammonia solution (cm^3)	Final volume (cm^3)	Concentration of Cu(II) ppm	Absorbance
1	0	5	100	-	
2	1	5	100	1	
3	2	5	100	2	
4	3	5	100	3	
5	4	5	100	4	
6	5	5	100	5	
7	6	5	100	6	
8	*unknown*	5	100	C$_x$	

Graph:

Plot a graph of absorbance against concentration of KMnO$_4$
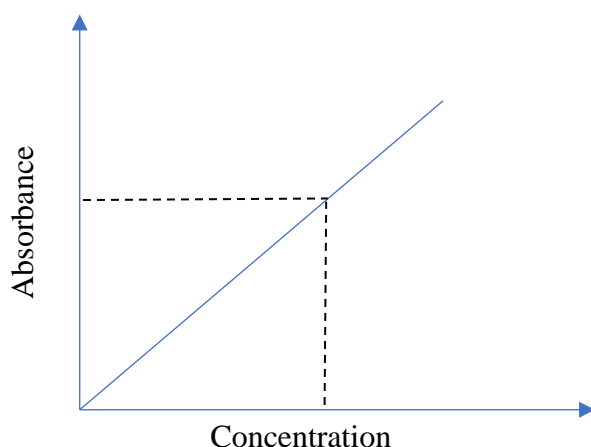
Calculations:

From the graph, concentration of Cu(II) in unknown solution is $C_x =$ _____ ppm

i.e. Concentration of Cu(II) in 10cm^3 of unknown solution $= C_x =$ _____ ppm

Concentration of Cu(II) in 100 cm^3 of unknown solution $= 10\ C_x =$ _____ ppm

Given Concentration of the unknown $=$ A ppm

Percentage error $= \dfrac{10\ C_x - A}{A} \times 100 =$ _____%

Result:

1. Concentration of Copper (II) ions in unknown solution = _____ ppm

2. Percentage Relative error =

MICROSCALE EXPERIMENTS IN CHEMISTRY

INTRODUCTION:

The rising cost of chemicals and decreased flow of funds are causing great concerns to the chemistry teachers. A tug - of - war had also been going on in recent years, to balance the budget of running practical courses and the standards of experiments to be carried out by the students. Since the economy is always the winner, the number of experiments had been the losers. A group of scientists from University of Pune and Fergusson College, Pune are striving hard to maintain the standards of experiments at a friendly budget ie., by carrying out the organic reactions in capillaries / tiles, using semimicro test tubes for heating experiments (instead of the conventional test tubes) and adopting to microscale preparations. Their attempts serve many purposes.

(i) cuts down the cost of chemicals.

(ii) experiments have become environment friendly.

(iii) less time consuming

(iv) less hazardous to the teachers, students and lab, assistants. What more it becomes affordable.

ANALYSIS OF AN ORGANIC COMPOUND

A) **Preliminary Tests:**

EXPERIMENT	OBSERVATION	INFERENCE
1. Test For Aromaticity: a. Substance is introduced into the flame using a nickel spatula.	i. Burns with a smoky flame ii. Burns with a non smoky flame	i.Presence of an aromatic compound ii.Presence of an aliphatic compound
b. Substance is added to a mixture of 3 drops of con.sulphuric acid & 3 drops of con. nitric acid and warmed on a water bath for about 10 minutes. The solution is poured, then into water.	An yellow solution or precipitate is formed	Presence of an aromatic compound The yellow colour is due to the formation of nitro compounds by the nitration of aromatic compounds.
2.Test For Unsaturation a. A drop of the substance is taken on a porcelin tile and add adrop of con. Bromine water to it.	i. Decolouration ii. Decoloration followed by tubidity	i. Presence of unsaturation ii. Presence of aniline/ phenol
b. To a pinch of substance add, a drop of dil. $KMnO_4$	Decolouration	Presence of unsaturation/ easily oxidisable compound
3. Solubility Tests: i. water	i. Soluble	i. Presence of urea, carbohydrates etc.

ii.5 % Na$_2$CO$_3$ iii.5% NaOH iv. 5% HCl	ii. Soluble iii. Soluble iv. Soluble	ii. Presence of acids iii. Presence of acids, phenols iv. Presence of amines
5. Action on Litmus: Moistened litmus paper is brought into contact with the substance	i. Blue litmus paper turns red ii. Red litmus paper turns blue iii. Neutral	i. Presence of acids, phenols ii. Presence of amines iii.Presence of carbohydrates, esters, carbonyls etc.
6. Action of Sulphuric Acid 5 mg. of the substance is warmed with 3 drops of con. H$_2$SO$_4$	Chars with a smell of burnt sugars	Presence of carbohydrate

B) Solubility test

EXPERIMENT	OBSERVATION	INFERENCE
1. About 5mg of the compound is taken on a watch glass. One or two drops of water are added. .	a. The compound partially dissolves. b. The mixture remains undissolved	Presence of carbohydrates, urea amine salts, salts of carboxylic acids, sulphanilic acid dicarboxlic acid etc b. Absence of all the above
2. In another watch glass 5mg of compound is taken and then tested with a drop of the saturated bicarbonate solution	Brisk effervescence. The solid acid reappears on addition of a drop of 8N HCl	Presence of an acid confirmed.

Theory:

The carboxylic acids react with bicarbonate to form sodium salts of the acids. Alkali metal salts being water soluble comes along with the aqueous layer. On acidification the free acid is generated as a solid.

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

$$RCOONa + HCl \longrightarrow RCOOH + NaCl$$

3. In a watch glass 5mg of compound is taken and then	Solubility observed and a Turbidity or oil is formed	presence of phenolic compound confirmed.

tested with a drop of 2N sodium hydroxide solution.	upon adding a drop of 8N HCl	

Theory:

Phenolic compounds react with sodium hydroxide to form sodium phenolate. Since alkali metal salts are soluble in water, phenolate ion comes along with the aqueous layer. Upon acidification, liberates the phenol.

$Ph\text{-}OH + NaOH \longrightarrow PhONa + H_2O$

$PhONa + HCl \longrightarrow PhOH + NaCl$

4. In another watch glass 5 mg of compound is taken and then tested with a drop of 2N hydrochloric acid solution.	Solubility observed and a Turbidity or oil is formed upon adding a drop of 10N NaOH.	presence of amino compound confirmed.

Theory:

The amines form amine hydrochlorides with hydrochloric acid. The hydrochloride being water soluble comes along with the aqueous layer. Upon neutralization the free amine is liberated.

$RNH_2 + HCl \longrightarrow RNH_2.HCl$

$RNH_2.HCl + NaOH \longrightarrow RNH_2 + NaCl + H_2O$

If all the above tests are negative then the given compound is **Neutral** in nature.

C) Detection of C, H, O, N, S and X⁻

(Lassaigne's Test) Preparation of Sodium fusion Extract: Two small cut pieces of sodium are fused in a semi-micro hard glass tube. About 5mg of the substance is added to it and fused again. The tube is cooled to room temperature. Keeping the tube in a slanting position 4 drops of water are added (the first drop of water is allowed to react with excess of sodium. The second drop is added after the initial reaction is over. Then the third and the fourth drops of water are added slowly). This is the sodium fusion extract. The following tests are performed with it.

a. Test for Nitrogen A drop of the extract is placed on a tile. A drop of a con. solution of $FeSO_4$ followed by a drop of 50% H_2SO_4 is added to it.	Prussian blue colour is obtained	Presence of nitrogen

b. Test for Halogens: A drop of the extract is treated with con. HNO₃ followed by a drop of AgNO₃ solution	i. Curdy white precipitate soluble in ammonium hydroxide ii. Pale yellow precipitate sparingly soluble in ammonium hydroxide iii. Yellow precipitate insoluble in ammonium hydroxide	i. Presence of chlorine ii. Presence of bromine iii. Presence of iodine
If halogen is present, About 5 mg of the substance is warmed with 3 drops of alcoholic AgNO₃ on a water bath and acidify with a drop of con. HNO₃.	i. Immediate formation of a precipitate ii. No precipitate	i. Presence of halogen in the side chain ii. Presence of halogen in the aromatic nucleus
c. Test for Sulphur: A drop of the extract is mixed with a drop of sodium nitroprusside on a tile.	Violet colour	Presence of sulphur

D) Determination of class of compound:
I.   Under C, H, (O) group:

1. Test for carboxylic acid: 1a. About 5 mg of the substance is treated with a drop of highly saturated NaHCO₃ solution on a tile	Brisk effervescence	Presence of carboxylic acid
1b. A drop of the substance is mixed with a drop of neutral FeCl₃ solution on a tile	i. Violet color ii. Flesh color	i. Presence of salicylic acid ii. Presence of phthalic acid
2. Test for ortho - dicarboxylic acid: About 5 mg. of the substance is heated with an equal amount of resorcinol and I drop of con. H₂SO₄ in a semi micro tube. Take a drop of it at the tip of a glass rod and dip it in very dil. NaOH in a semi micro tube.	Green fluorescence	Presence of o- dicarboxylic acid

3. Test for Phenol: **a. Neutral Ferric chloride test** A drop of the substance is mixed with a drop of neutral $FeCl_3$ solution on a tile.	Violet colouration	Presence of phenol
b. Phthalein Reaction: About 5 mg of the Substance is heated with about 10 mg of phthalic anhydride and a drop of con. sulphuric acid in a semimicro tube. The solution is cooled and diluted with about 1 ml of water. A drop of the solution is mixed with a drop of 50%, sodium hydroxide solution on a tile.	Red, blue or green color	Presence of phenol
c. Liebermann's reaction : About 5 mg of the substance is heated with about 5 mg of sodium nitrite and 2 drops of con. sulphuric acid in a semi micro tube. It is cooled and diluted with about 1 ml of water. A drop of it is placed on a tile and mixed with a drop of 10% sodium hydroxide solution.	A bluish green color is produced	Presence of phenols
4. Test for Carbohydrates: (Molisch's test) A drop of an alcoholic solution of α- naphthol is mixed with a drop of aqueous solution of the substance on a tile and was mixed with a drop of con. Sulphuric acid.	A deep violet color	Presence of sugars
5a. Test for aldehydes / ketones: Borsche's reagent test:	Red orange precipitate	

A drop of the substance (if it is liquid) or a drop of an alcoholic solution is placed on a tile. A drop of the con. Solution of 2,4- DNP (Borsch reagent) is added to it.		Presence of aldehydes/ ketones
5b. Schiff's reagent test: A drop of the substance is mixed with a drop of Schiff's reagent	Pink colour	Presence of aldehyde
5c. 2 drops or about 5 mg of the substance is mixed with 2 drops of Fehling A and 2 drops of Fehling B solutions in a semimicro test tube.It is heated in a water bath for about 5 minutes.	Red precipitate	Presence of aldehyde/ Reducing sugars
6a. Test for ester: About 2 drops of the substance is heated with 2 drops of methanolic solution of hydroxylamine hydrochloride and 2 drops of 50% NaOH and cooled. A drop of it is placed on a tile. It is mixed with dil. HCl and neutral $FeCl_3$, a drop of it each.	Violet color	Presence of ester
6b. drops of the substance is heated with 2 drops of 50% NaOH solution, till it is dissolved. Cool it and then add 4 drops of 50% HCl.	White precipitate	Presence of ester
7a. Test for alcohols: Add a small piece of Na metal to 2 drops of the substance.	Effervescence	Alcohols present
7b. A drop of the substance is added to a drop of acetyl chloride in fume hood.	Violent reaction with evolution of fumes.	Alcohols present

8. Test for Hydrocarbons: With 5mg each of the substance and picric acid prepare a saturated solution in alcohol separately (add 3 or 4 drops of alcohol). Mix The solutions, heat and Cool.	Yellow precipitate	Presence of hydrocarbons

II. Under C, H, (O), N group:

1. Test for primary Amines : Dye test : A drop of the substance is placed on a tile. A drop of dil. hydrochloric acid is added to it. A drop of saturated sodium nitrite solution followed by a drop of β - naphthol dissolved in 25% NaOH is added to it.	Red azo dye	Presence of aromatic primary amine
2.Test for Secondary Amines: 2 drops of substance is mixed with 2 drops of dil. Hydrochloric acid and 2 drops of saturated solution of sodium nitrite in a semimicro tube. A drop of con. sulphuric acid and a drop of phenol are added. Heated for a minute. A drop of this solution is mixed with a drop of dil. NaOH on a tile.	A bluish green color is produced	Presence of secondary amine
3. Test for tertiary amine Dye test : 2 drops of benzaldehyde and 4 drops of the substance are heated with 1 drop of con.sulphuric acid and 50 mg of lead dioxide. A drop of this solution is mixed with a drop of dil. HCl on a tile.	Malachite green dye is formed	Presence of tertiary amine.
4a. Test for nitro group: Reduction to amines :		

About 3 drops of the substance is reduced with 3 drops of con. hydrochloric acid and metallic tin or zinc in a semimicro tube, by heating for about 5 minutes. with a drop of the supernatant liquid dye test is performed on a tile. (as under test for amines)	Red azo dye	Presence of nitro group
4b. Mulliken - Barker's test: About 3 drops of the substance and 3 drops of alcohol are boiled with 3 drops of calcium chloride solution and a pinch of zinc dust. Heated to boiling and cooled. A drop of it is mixed with a drop of Tollen's reagent on a tile.	A black precipitate	Presence of nitro group
5. Test for Anilide: About 10 mg of the substance is heated strongly in a dry semi micro tube with soda lime. The resulting vapour is collected in another semi micro tube containing 5 drops of dil. hydrochloric acid. With a drop of the acid solution dye test is performed	Scarlet Red dye	Presence of anilide
6. Biuret test: About 5 mg of the substance is heated strongly in a dry semi micro tube to its melting point. Cooled and the residue is dissolved in 3 drops of water. A drop of it is mixed with a drop of dil. CuSO4 and with a drop of dil. NaOH solution on a tile.	A violet colour	Presence of diamide
7. Test for Aromatic monoamide: About 5 mg of the substance is heated with 5 drops of 25% NaOH solution and cooled. A	White precipitate	Presence of monoamide

drop of it is mixed with a drop of con. HCl		

III.   Under C, H, (O), N, S group:

1a. Test for thiourea: About 5 mg of the substance is heated with 5 drops of dil. NaOH solution and cooled. A drop of it is mixed with a drop of lead acetate solution on a tile.	A black / brown precipitate	Presence of thiourea
1b. 5 mg of the substance is heated in a dry semi micro tube until it melts. It is cooled and the residue is dissolved in 4 drops of water. A drop of it is mixed with a drop of neutral $FeCl_3$ solution on a tile.	Blood red coloration	Presence of thiourea