SOPs/ INSTRUCTION MANUAL

1) Preparation of solution of different concentration from given stock solution.

If you have a concentrated stock solution of known concentration, you can prepare a working solution of specific concentration and volume by diluting the stock solution.
Use the following formula to calculate the volume of stock solution required to prepare a diluted solution

$$C_f \times V_f = C_S \times V_S$$

C_f: Concentration of diluted solution
V_f: Volume of diluted solution
C_S: Concentration of stock solution
V_S: Volume of stock solution
Example: The concentration of stock solution is given in x fold (times) e.g. 10X stock solution
For example, you want to prepare 100 ml of working solution (after dilution) from 10X stock solution
C_f: 1 X
V_f: 100 ml
C_S: 10 X
V_S: ? ml (to be calculated)
Formula: $C_f \times V_f = C_S \times V_S$

Place all values in formula:
$1 \times 100 = 10 \times V_S$
$Vs = 1 \times 100/10$
$V_S = 10$ ml
So you need to take 10 ml of 10 X stock solution to prepare a 100 ml working (diluted) solution.
How much solvents do you need to add?
The final volume of the working (diluted) solution: 100 ml
The volume of stock solution to be taken: 10 ml
The volume of water to be added: 100 – 10 = 90 ml
So you need to mix 10 ml of 10X stock solution and 90 ml water to obtain 100 ml of working (diluted) solution.
Example: When the concentration of stock solution concentration is given in a concentration unit e.g. 1 M stock solution
For example, you want to prepare 100 ml of 1 mM EDTA solution from 0.5M EDTA solution
C_f: 1 mM
V_f: 100 ml
C_S: 0.5 M
V_S: ? ml (to be calculated)
Tip: Stock concentration is given in M (molar) and working concentration is given in mM (milliMolar). Convert Stock concentration Molar to millimolar.
1 M = 1000 mM
Now the values are:
C_f: 1 mM
V_f: 100 ml
C_S: 500 mM
V_S: ? ml (to be calculated)
Place all values in formula:

$C_f \times V_f = C_S \times V_S$
$1 \times 100 = 500 \times V_S$
$Vs = 1 \times 100/500$
$V_S = 0.2$ ml
So you need to take 0.2 ml of 0.5M EDTA solution to prepare 100 ml of 1 mM EDTA solution.
How many solvents do you need to add?
The final volume of the working solution: 100 ml
The volume of stock solution to be taken: 0.2 ml
The volume of water to be added: 100 – 0.2 = 99.8 ml
So you need to mix 0.2 ml of 0.5M EDTA solution and 99.8 ml water to obtain 100 ml of 1 mM EDTA solution.

2) To separate cations of group I (Pd^{2+}, Ag^{+} , Hg_2^{2+}) by using paper chromatography.

Apparatus: Beakers, Stirrer, Watch glass, Capillary tube Whatmann filter paper, measuring cylinder etc.

Chemicals required: Distilled water, Acetone, Lead nitrate, Silver nitrate and mercurous nitrate.

Theory: Paper chromatography can be considered to be a type of partition chromatography where the partition occurs as a consequences solid-liquid or ion exchange equilibria.Two types of forces operate when drop of solution is applied on the filter paper and treated with a solvent.

Propelling forces: It tries to drag the substances in the direction of flow of solvent. This depend upon:

- *The rate of flow of the solvent.*
- *The solubility of the substances in the solvent substances have different solubilities nature. The component having higher solubility will move rapidly along the strip of the filter paper than the less soluble component.*

Retardation forces: Retarding force tries to drag the substance behind toward its point of applied cation. This retardation depends on the absorption and partition. The amount of solute in each solvent depends on relative solubility of the substances in the two solvents. This leads to the partition coefficient at the equilibrium and the contribution of R_f.

Retardation factor R_f value: The relative rate of the movement of solvent and solute is expressed by a term R_f value.

It is defined as the ratio of the distance travelled by the component at its point of maximum concentration to distance travelled by the solvent.

$$R_f = \frac{\text{Distance travelled by sample}}{\text{Distance travelled by solvent}}$$

Procedure:

1. Take a pinch of each compound in a beaker.
2. Now prepare a mixture of these compound by dissolving them in minimum quantity of solvent prepared above in a watch glass.
3. Take whatmann filter paper and cut it into rectangular strips.
4. Draw a straight line about 1-1.5 cm above on lower end.
5. Insert a drop of mixture of indicators with the help of capillary tube at the centre of the line drawn.
6. Place this strip in the beaker containing the solvent such that the mark must not be dip in the solvent and cover the beaker with watch glass.
7. After a little time the solvent starts rising up due to absorption phenomenon and mixture of indicators will separate out forming an orange red, orange spot and the yellow spot on the strip.
8. Measure the distance of these spots and of solvent front from the lower line.
9. Now calculate the R_f value for these measured distances.

General calculations:

$$R_f = \frac{\text{Distance travelled by sample}}{\text{Distance travelled by solvent}}$$

R_f value of yellow spot

$$R_f = \frac{\text{Distance travelled by yellow spot}}{\text{Distance travelled by solvent}}$$

R_f value of orange spot

$$R_f = \frac{\text{Distance travelled by orange spot}}{\text{Distance travelled by solvent}}$$

R_f value of orange red spot

$$R_f = \frac{\text{Distance travelled by orange red spot}}{\text{Distance travelled by solvent}}$$

Result:

R_f value of yellow spot = ----------

R_f value of orange spot = ----------

R_f value of orange red spot = ----------

3) QUANTITATIVE ANALYSIS –DETECTION OF ELEMENTS

As we know all organic compounds contain carbon and hydrogen. Whether oxygen is present or not does not make any contribution for detection and no special test is suggested. In fact the presence of Nitrogen, Sulphur and Halogens has to be detected.

Preparation of Lassaigne's extract or Sodium fusion extract

Take a freshly cut small piece of dry sodium metal in a fusion tube and heat gently. The sodium metal looks like a globule of mercury. Put a small quantity of organic compound under examination into the tube. Liquids are introduced into the tube with the help of a small dropper. Heat the fusion tube slowly at first, then strongly until it is red hot and maintain it at this temperature for a minute or two. Place the red hot end of fusion tube in 5-10 ml of distilled water taken in a china dish. Crush the content of the glass tube with a glass rod and when the reaction with left-over sodium, if any, has subsided, heat the contents of the china dish to boiling. Remove the insoluble matter by filtration. The filtrate known as Lassaigne's extract or Sodium extract, contain nitrogen, sulphur or halogens of the compound in the form of sodium cyanide, sodium suphide or sodium halide due to the following reaction.

$$Na + C + N \rightarrow NaCN$$

From organic compound

$$2Na + S \rightarrow Na_2S$$

From organic compound

$$Na + C + N + S \rightarrow NaCNS$$ (if N and S both are present sodium sulphocyanide)

from organic compound

$$Na + x \rightarrow NaX$$ (X= Cl ,Br ,I)

DETECTION OF NITROGEN

1. **SODA LIME TEST**. Heat the small quantity of the organic compound with soda lime (NaOH + CaO) in a test tube. Evolution of ammonia indicates the presence of nitrogen. Ammonia evolved gives white fumes with hydrochloric acid and brown ppt. When passed through Nessler's reagent solution.

$$CH_3CONH_2 + NaOH \xrightarrow{Cao} CH_3COONa + NH_3\uparrow$$

Acetamide

2. **Lassaigne's test** .(i) Take 2-3ml of lassaigne's extract in a test tube and test if it is alkaline to litmus. If it is not, make it alkaline by adding a few drops of sodium hydroxide.

(ii) Add 2ml of freshly prepared $FeSO_4$ solution when dirty green precipitate of $Fe(OH)_2$ is formed. Boil the reaction mixture for 1-2 minutes and cool.

(iii) Add 2-3 drops of ferric chloride solution to the cooled solution and acidify the resulting solution with dilute hydrochloric acid.

A prussion blue or green colouration confirms the presence of nitrogen and a blood red colouration indicates the presence of nitrogen and sulphur both.

$$Na + C + N \rightarrow NaCN$$

$$FeSO4 + 2NaOH \rightarrow Fe(OH)_2 + Na_2SO_4$$

(Green ppt.)

$$FeSO_4 + 6NaCN \rightarrow Na_4[Fe(CN)_6] + Na_2SO_4$$

$$3\ Na_4[Fe(CN)_6] + 4FeCl_3 \rightarrow Fe_4[Fe(CN)_6]_3 + 12NaCl$$

(Prussian blue)

If nitrogen and sulphur both are present, as in thiourea a blood red colouration is produced due to the following reaction.

$$3NaCNS + FeCl_3 \rightarrow Fe(CNS)_3 + 3\ NaCl$$

(blood red colour)

However, absence of blood red colour does not mean that sulphur is always absent .The NaCNS formed is decomposed by excess of sodium and no blood red colouration.

$$NaCNS + 2Na \rightarrow NaCN + Na_2S$$

DETECTION OF SULPHUR

1. **LEAD ACETATE TEST**: Take 2ml of lassaigne's filtrate in a test tube .Acidify it with 2-3 drops of acetic acid and add lead acetate solution. A black ppt. of lead sulphide indicates the presence of sulphur.

$$Pb(CH_3COOH)_2 + Na_2S \rightarrow PbS \downarrow + 2CH_3COONa$$

Lead acetate (black ppt.) sodium acetate

2. **SODIUM NITROPRUSSIDE TEST**: Take 2ml of sodium extract and add to it a few drops of freshly prepared dilute solution of sodium nitroprusside. A deep violet colour indicates the presence of sulphur in the given compound.

$$Na_2S + Na_2[Fe(CN)_5NO] \rightarrow Na_4[Fe(CN)_5\ NOS]$$

(Violet colouration)

DETECTION OF HALOGENS

1. **SILVER NITRATE TEST**

Take 3ml of Lassaigne's filtrate in a test tube and add to it 1ml of conc. nitric acid. Boil the contents for a minute, cool and add to silver nitrate solution.

(a)White ppt. Soluble in ammonium hydroxide indicates chloride ions and hence chlorine element in the organic compound.

(b) Pale yellow ppt. Sparingly soluble in ammonium hydroxide indicates bromide ions and hence bromine element in the compound.

(c) Yellow ppt. Insoluble in ammonium hydroxide indicates iodine ions and hence iodine element in the organic compound.

$$NaCl + AgNO_3 \rightarrow AgCl \downarrow + NaNO_3$$

(White ppt.)

$$AgCl + 2\ NH_4OH \rightarrow [Ag(NH_3)_2]Cl + 2H_2O$$

Soluble

$$NaBr + AgNO_3 \rightarrow AgBr \downarrow + NaNO_3$$

(Pale yellow)

$$NaI + AgNO_3 \rightarrow AgI \downarrow + NaNO_3$$

(Yellow)

The tests of N, S and halogens are summarised in table below

EXPERIMENT	OBSERVATION	INFERENCE
1. **Prepration of Lassaigne's extract** Fused the given organic compound with freshly cut small piece of sodium metal in a fusion tube till it becomes red hot. Pluged the red hot end of fusion tube into china dish containing 10 to 15 ml of distilled water. Repeat two more fusions. Boiled for 5 minutes and filtrated.	(i) Residue (ii) Filtrate	Rejected L.E
2. **Test of Nitrogen** To a portion of L.E in a test tube added 2 drops of NaOH solution. Then added 1-2 ml of freshly prepared ferrous sulphate solution. Bioled for 2-3 minutes, cooled and added a few drops of $FeCl_3$ acidified with conc. HCl.	A Prussian blue colour Blood red colour	Nitrogen present N and S both are present
3.**Test for sulphur** (i) Lead acetate test Acidified second portion of L.E with dilute acetic acid and added Lead acetate solution.	Black ppt.	Sulphur present
(ii) Sodium Nitroprusside test To third portion of L.E added sod.	Violet colour	Sulphur present

Nitroprusside solution. 4. **Tests for halogens** Silver nitrate test Acidified 2-3 ml of L.E with conc. HNO_3 and boiled for 1-2 minutes .Cooled and added silver nitrate solution.	(i)A curdy white ppt. Soluble in excess of ammonium hydroxide (ii)A pale yellow ppt. Partially soluble in NH_4OH (iii)A yellow ppt. Insoluble in NH_4OH	Chlorine present Bromine present Iodine present

DETECTION AND IDENTIFICATION OF FUNCTIONAL GROUP

The molecules of simple organic compounds may be regarded to be derivatives of hydrocarbons in which one or more of the hydrogen atoms have been replaced by different functional groups eg.-OH ; >C=O ; -COOH ; $-NH_2$ etc.

For the sake of convenience the organic compounds are classified into the following four types depending upon the presence of elements nitrogen, halogen and sulphur.

TYPE 1.TESTS FOR COMPOUNDS CONTAINING CARBON AND HYDROGEN (WITH OR WITHOUT OXYGEN)

Organic compounds which fall in this type are:

(I) CARBOXYLIC GROUP

EXPERIMENT	OBSERVATIONS	INFRENCE

1. **LITMUS TEST** Dissolve a pinch of the organic compound in 2ml of water in a test tube and add to it 2-3 drops of blue litmus solution.	Blue litmus turns red	Presence of a carboxylic or a phenolic group.
2.**SODIUM BICARBONATE TEST** Dissolve a pinch of the organic compound in water in a test tube. Now add to it a few drops of 5% aqueous $NaHCO_3$ solution with a dropper .	Effervescence due to the evolution of carbon dioxide	Presence of a carboxylic group
3. **ESTER FORMATION TEST** Heat a pinch of the organic compound in a dry test tube with 2-3 ml of absolute ethyl alcohol and 1-2 drops of conc. H_2SO_4. Cool and pour the contents of the tube into an aqueous solution of sodium carbonate.	Pleasent fruity smell	Presence of an ester and hence, the carboxylic group
4. **FERRIC CHLORIDE TEST** Add a pinch of the organic compound to 2-3 ml of distilled water taken in a test tube . now add a few drops of ammonium hydroxide	Coloured ppt is obtained	Presence of carboxylic group.

solution to make it just alkaline and shake .Boil off excess of ammonia .Cool and add a few drops of neutral ferric chloride solution to it .		

EXPLANATION

SODIUM BICARBONATE TEST

$RCOOH + NaHCO_3 \rightarrow RCOONa + CO_2 + H_2O$

ESTER FORMATION TEST

$RCOOH + CH_3CH_2OH \xrightarrow{H^+} RCOOCH_2CH_3 + H_2O$

FERRIC CHLORIDE TEST

$RCOOH + NH_4OH \rightarrow RCOONH_4 + H_2O$

$3RCOONH_4 + FeCl_3 \rightarrow (RCOO)_3Fe + 3NH_4Cl$

$(RCOO)_3Fe + H_2O \rightarrow Fe(OH)(OOCR)_2 + RCOOH$

Name of the carboxylic acid	**Colouration or ppt. In the $FeCl_3$ test**
Acetic acid	Deep red coloration which changes to reddish brown ppt. On heating
Oxalic acid	Light yellow ppt.
Benzoic acid	Buff –coloured ppt.
Phthalic acid	Brownish –buff coloured ppt.
Cinnamic acid	Light yellow ppt.
Succinic acid	Buff ppt. Soluble in dil.HCl

Distinction between Aliphatic and Aromatic acids –Sodium hydroxide test: Add a pinch of the organic acid to a test tube containing 2-3ml of aq. NaOH solution and shake .The acid rapidly dissolve due to the formation of the corresponding sodium salt.

$$RCOOH + NaOH \rightarrow RCOONa + H_2O$$

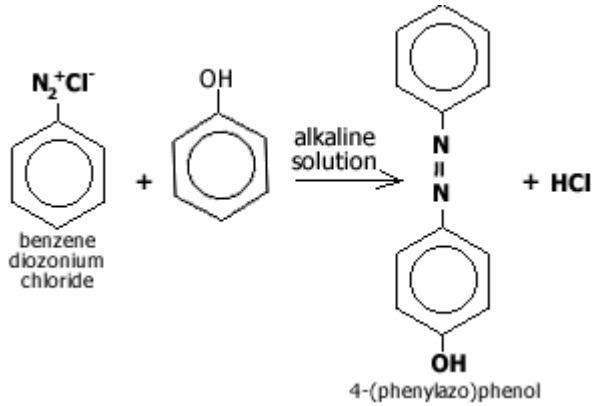
Acidify the above solution with dil.HCl .if a white ppt. Is obtained, it shows that the given compound is an aromatic acid otherwise aliphatic acid.

PHENOLIC GROUP

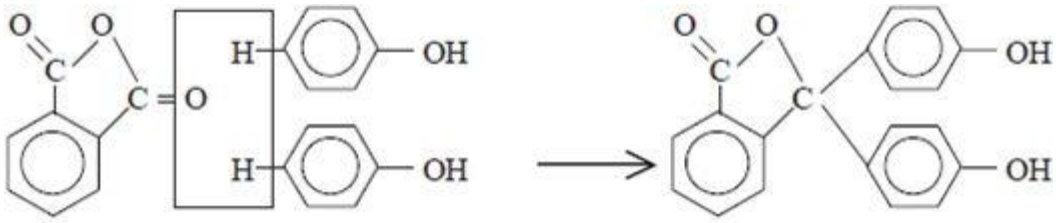
EXPERIMENT	OBSERVATION	INFERENCE
1. **LITMUS TEST** Dissolve a pinch of the organic compound in a 2ml of water in a test tube and add to it 2-3 drops of the blue litmus solution.	Blue litmus turns red	Presence of a carboxylic or a phenolic group.
2.**FERRIC CHLORIDE TEST** To an aqueous solution of the organic compound ,add 4-5 drops of neutral ferric chloride solution .	Appearance of a violet ,blue ,green or red colouration	Presence of phenolic group
3. **AZO DYE TEST** Dissolve a pinch of organic compound in dil. NaOH solution. In another test tube take 0.5 ml of aniline and dissolve in dil.HCl. Cool two tubes in ice –cold water. Add	Brilliant red dye	Presence of phenolic group.

solid $NaNO_2$ to aniline hydrochloride solution. Finally add the alkaline phenolic solution to the diazotized mixture solution. 4. **PHTHALEIN TEST** Take 0.2g of organic compound ,0.5g of phthalic anhydride and 2-3 drops of conc. H_2SO_4. Heat for about 1 minute. Cool and pour the reaction mixture into 15ml of dilute NaOH solution taken in a beaker.	Intense green, purple, blue or red colour obtained.	Phenolic group present.

EXPLANATION.DYE FORMATION TEST:

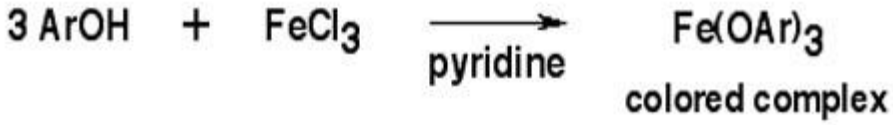


2. **PHTHALEIN TEST**: When phenols are heated with phthalic anhydride in the presence of conc. H_2SO_4, they undergo condensation to form phthalein dyes which give characteristic colour in NaOH solution.



Phenolphthalein

3. **FERRIC CHLORIDE TEST**:



ALCHOLIC HYDROXIDE GROUP

Alcohols are classified as primary (1^0), secondary (2^0) and tertiary (3^0) depending upon wheather –OH group is attached to 1^0, 2^0, 3^0 carbon atom respectively.

Alcohols are neutral towards litmus and also do not produce effervescence with $NaHCO_3$ solution .these are generally indicated by the following tests.

EXPERIMENT	OBSERVATION	INFERENCE
1. SODIUM METAL TEST Add freshly cut piece of sodium metal to 0.5 ml of the organic liquid in a dry test tube. If the organic compound is a solid, prepare its solution in dry benzene or ether and then add to it a freshly cut piece of sodium metal.	Brisk effervescence due to evolution of hydrogen.	Presence of an alcoholic hydroxide group.

2. **ACETYL CHLORIDE TEST**: To about 0.2ml of the organic compound, add 0.5ml of acetyl chloride dropwise.	HCl gas is evolved which is detected by its pungent smell and dense white fumes with NH_4OH.	Presence of an alcoholic hydroxide group.
3. **ESTER TEST**: Heat about 1.0g of the compound with 1ml each of glacial acetic acid and conc.H_2SO_4.	A pleasant fruity odour.	Presence of an alcoholic hydroxyl group.

SODIUM METAL TEST:

$$2ROH + 2Na \rightarrow 2RONa + H_2$$

ACETYL CHLORIDE TEST:

$$ROH + CH_3COCl \rightarrow RCOOCH_3 + HCl$$

ESTER TEST:

$$ROH + CH_3COOH \rightarrow CH_3COOR + H_2O$$

DISTINCTION BETWEEN 1^0 ,2^0 ,3^0 ALCOHOLS

LUCAS TEST:

Take about 1ml of alcohol in a test tube, add 8-10 drops of Lucas reagent. Shake the mixture and observe

(i) If cloudiness appears immediately, it indicates tertiary alcohol.

(ii) If cloudiness appears within five minutes, it indicates secondary alcohol.

(iii) If cloudiness appears only upon heating, it indicates primary alcohol.

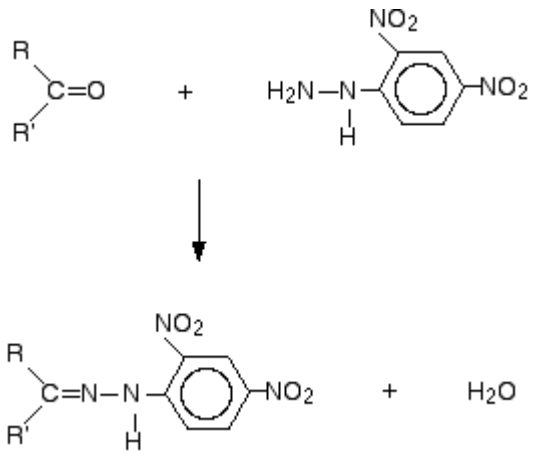
ALDEHYDES AND KETONE GROUP:

Both aldehydes and ketone contain the (>c=o) group in their molecules and are indicated by the following tests.

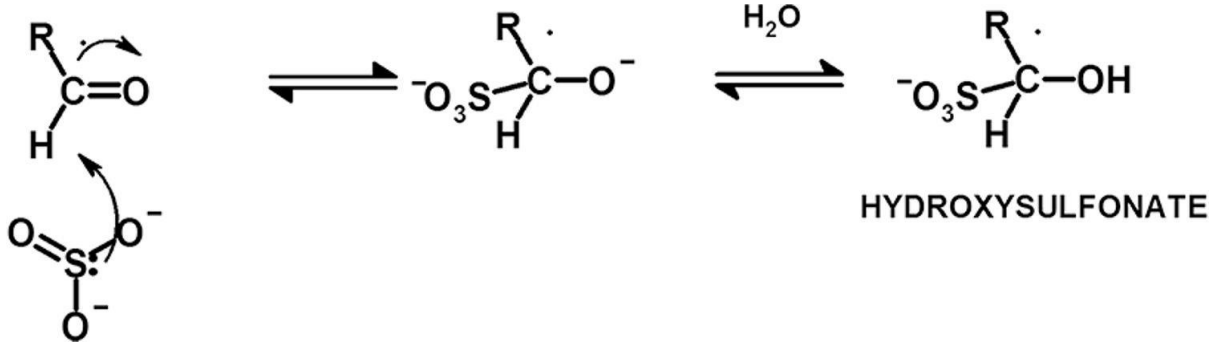
EXPERIMENT	OBESERVATION	INFRENCE
1. **2,4-DINITROPHENYLHYDRAZINE TEST OR BRADY'S TEST**: Dissolve 0.1g of the organic compound in 2ml of alcohol and add to it 1-2ml of 2,4 dinitrophenylhydrazine reagent and shake well.	Crystalline yellow, orange or red ppt.	Presence of the carbonyl group.
2. **SODIUM BUSULPHITE TEST**: Shake about 0.2g or 0.2ml of the compound with 1ml of a saturated solution of $NaHSO_3$.	White ppt.	Presence of the carbonyl group.
3.**TOLLEN'S REAGENT TEST**: Shake a pinch of the organic compound to the freshly prepared Tollen's reagent keep the tube in briskly boiling water for 2 minutes.	Grey or brown ppt .is formed silver mirror is obtained along the sides of the test tube.	Presence of an aldehydic group.

4. **FEHLING SOLUTION TEST**: Add a pinch of the organic compound to 1-2 ml freshly prepared Fehling solution and shake well. Keep the test tube in briskly boiling water for about 2 minutes.	Red ppt. Of Cu_2O.	Presence of an aldehydic group.

2,4-DINITROPHENYLHYDRAZINE OR BRADY'S TEST:



SODIUM BISULPHITE TEST :



TOLLEN'S REAGENT TEST:

$$AgNO_3 + NaOH \rightarrow AgOH + NaNO_3$$

$$AgOH + NH_4OH \rightarrow [Ag(NH_3)_2]^+OH^- + 2H_2O$$

Tollen's reagent

$$RCHO + 2Ag(NH_3)_2^+ + 3OH^- \rightarrow RCOO^- + 4NH_3 + 2Ag\downarrow$$

silver mirror

FEHLING SOLUTION TEST:

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

$$Cu(OH)_2 \rightarrow CuO + H_2O$$

$$RCHO + 2CuO \rightarrow RCOOH + Cu_2O$$

Red ppt.

This test is given by only aliphatic aldehydes and hence can be used to distinguish between aliphatic and aromatic aldehydes.

TEST FOR KETONIC GROUP

EXPERIMENT	OBSERVATION	INFRENCE
1. **2,4-DINITROPHENYLHYDRAZINE TEST OR BRADY'S TEST**: Dissolve 0.1g of the organic compound in 2ml of alcohol and add to it 1-2ml of 2,4 dinitrophenylhydrazine reagent and shake well.	Crystalline yellow, orange or red ppt.	Presence of the carbonyl group
2. **SODIUM BUSULPHITE TEST**: Shake about 0.2g or 0.2ml of the compound with 1ml of a saturated solution of $NaHSO_3$.	White ppt.	Presence of the carbonyl group.
3. **SODIUM NITROPRUSSIDE TEST**: Take about about 1ml of sodium nitroprusside solution and	Red colouration	Presence of ketonic group

make it alkaline by the addition of a few drops of sodium hydroxide solution. Now add a pinch of the organic compound to it and shake well. 4. **IODOFORM TEST**: Warm the given compound with iodine and 5% NaOH Solution in a boiling water bath.	Yellow ppt.	Presence of ketonic group

SODIUM NITROPRUSSIDE TEST:-

$$CH_3COCH_3 + OH^- \rightarrow CH_3COCH_2^- + H_2O$$

$$[Fe(CN)_5NO]^{2-} + CH_3COCH_2^- \rightarrow [Fe(CN)_5NOCH_3COCH_2)]^{3-}$$

Red

IODOFORM TEST

$$R\text{-}CO\text{-}CH_3 + 3I_2 + 3NaOH \rightarrow R\text{-}CO\text{-}Cl_3 + 3NaI + 3H_2O$$

$$R\text{-}CO\text{-}Cl_3 + NaOH \rightarrow CHI_3\downarrow + RCOONa$$

iodoform(yellow ppt)

ESTER GROUP

EXPERIMENT	OBSERVATION	INFRENCE
1. **PHENOLPHTHALEIN TEST**: Dissolve 0.2g or 0.2ml of the organic compound in 3-4ml of ethyl alcohol in a test tube and add to it, 1 drop of phenolphthalein and 2-3 drops of 5% aq. NaOH solution till a permanent pink colour is obtained .shake well	Pink colour disappears	Ester group present.

and keep the test tube in boiling water for a few minutes. 2.**HYDROXAMIC ACID TEST**: Dissolve 0.2ml or 0.2g of the organic compound in 5ml of 95% alcohol. Add 0.2g of hydroxylamine hydrochloride to it and shake well to make a clear solution. Now add 5ml of 10% NaOH to make the solution alkaline. Boil the reaction for about a minute, cool and acidify with dil. HCl. Then add a few drops of $FeCl_3$ solution.	Deep red or violet coloration	Ester group present

PHENOLPHTHALEIN TEST

$$C_6H_5COOC_2H_5 + NaOH \rightarrow C_6H_5COONa + C_2H_5OH$$

HYDROXAMIC ACID TEST

$$NH_2OH.HCl + NaOH \rightarrow NH_2OH + NaCl + H_2O$$

$$R\text{-}CO\text{-}OR' + NH_2OH \rightarrow R\text{-}CO\text{-}NHOH + R'OH$$

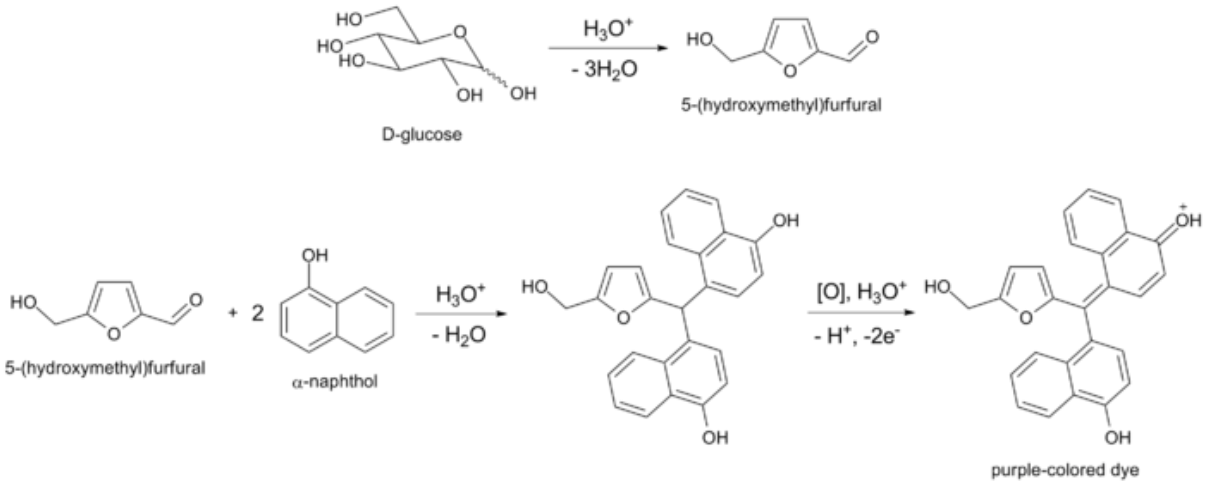
$$3R\text{-}CO\text{-}NHOH + FeCl_3 \rightarrow (RCONHO)_3Fe + 3HCl$$

CARBOHYDRATE GROUP:

This class of organic compound contains monosaccharides, disaccharides and polysaccharides. Except for cellulose and starch, they are highly soluble in water. However, all of them are insoluble in ether. Carbohydrates are detected by the following tests.

EXPERIMENT	OBSERVATION	INFERENCE
1. **DRY HEATING TEST**. Heat a pinch of the organic compound in a dry test tube.	Charring occurs with the characteristic smell of a burnt sugar.	Carbohydrates group present.
2. **Conc. H_2SO_4 TEST** Heat a pinch of the organic compound with 2ml of conc.H_2SO_4.	Charring occurs with the characteristic smell of a burnt sugar.	Carbohydrates group present .
3. **MOLISCH TEST** Dissolve about 0.2g of the organic compound in 1ml of water and add to it 4-5 drops of 10% alcoholic solution of α-naphthol. Shake the solution well. Now add carefully about 1ml of conc. H_2SO_4 along the sides of the test tube.	Deep violet ring appers at the junction of the two layers.	Carbohydrates group present .

MOLISCH **TEST**:



DISTINCTION BETWEEN REDUCING AND NON-REDUCING SUGARS

EXPERIMENT	OBSERVATION	INFERENCE
1. **TOLLEN'S REAGENT TEST** Take 2ml of freshly prepared Tollen's reagent in a test tube and add to it a pinch of the carbohydrate. Place the test tube in briskly boiling water for 2 minutes.	A silver mirror is obtained along the sides of the test tube.	Presence of a reducing sugar
2. **FEHLING SOLUTION TEST** Mix equal volumes of Fehling solution A and Fehling solution B. Now add a pinch of the sugar to this deep blue solution. Place the test tube in briskly boiling water for about 2 minutes.	Red ppt. of Cu_2O	Presence of a reducing sugar.
3. **BENEDICT'S**		

SOLUTION TEST Take 2ml of Benedict's solution in a test tube and to it 0.2g of the organic compound. Boil for 2 minutes and then cool.	Red ppt. Of Cu_2O	Presence of a reducing sugar

Chemistry of the tests

1. **Fehling solution test**

On mixing equal volume of Fehling A($CuSO_4$ Solution)and Fehling solution B (Roschelle salt in NaOH solution), a deep blue solution is obtained . on heating with reducing sugars, a red precipitate of cuprous oxide is obtained.

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

$$Cu(OH)_2 \rightarrow CuO + H_2O$$

2. **Tollen's reagent test**

On heating with reducing sugars, Tollen's reagent gives a deposit of silver which appears as mirror.

$$2AgNO_3 + NH_4OH \rightarrow AgOH + 2NH_4NO_3$$

$$2AgOH \rightarrow Ag_2O + H_2O$$

ETHER GROUP

Ethers can be regarded as the derivative of water in which the hydrogen atoms are replaced by aliphatic or aromatic groups. Ethers are volatile inflammable liquids having pleasant odour. They are soluble in conc. H_2SO_4. This solubility distinguishes ethers from hydrocarbons.

IDENTIFICATION TESTS FOR ETHERS

EXPERIMENT	OBSERVATION	INFRENCE
1. **Iodine test** Dissolve 1ml of compound in 3ml benzene, then add 3ml of dilute solution of iodine in	Brown coloured solution	Ether present

benzene.		
2. **Feigl's test** Take 2ml of the compound in a test tube and keep a filter paper moistened in cupric acetate and benzidine hydrochloride on the mouth of the test tube .Heat the contents for 4-5 minutes.	Deep blue colour appears on the filter paper	Ether present

AMIDES (-CO-NH_2 GROUP)

Amides are derivatives of carboxylic acids in which –OH group is replaced by amino group. These are soluble in water due to hydrogen bonding.

IDENTIFICATION TESTS OF AMIDES

EXPERIMENT	OBSERVATIONS	INFRENCE
1.**Sodium hydroxide test** Heat a pinch of organic compound with NaOH solution .added dil.HCl to above solution.	Vapour with ammonical smell evolved (i)white ppt. Formed (ii)No white ppt. Formed (iii)CO_2 gas evolved	Amide present Aromatic amide present Aliphatic amide present Urea present
2. **Nitrous acid test** Dissolve a pinch of organic compound in dil.HCl. In another test tube dissolve 1.0g of $NaNO_2$ in 5.0 ml of water .cool the solutions in ice and mix them. 3. **Test for aromatic amides** To an aqueous solution of	Brisk effervescence of nitrogen gas Immediate bluish-red	Amide present Aromatic amide present

compound add 5-7 drops of 6% H_2O_2 and boil the contents .cool and add 1-2 drops of 5% $FeCl_3$ solution.	colouration in cold.	

Chemistry of tests for amide group

1. **Sodium hydroxide test**

$$RCONH_2 + NaOH \rightarrow RCOONa + NH_3$$

On acidification with dil.HCl a ppt of insoluble aromatic acid is formed

$$ArCOONa + HCl \rightarrow ArCOOH \downarrow + NaCl$$

2. **Nitrous acid test**

$$NaNO_2 + HCl \rightarrow HNO_2 + NaCl$$

$$RCONH_2 + HNO_2 \rightarrow RCOOH + N_2 + N_2O$$

3. **Test for aromatic amides**

$$ArCONH_2 + H_2O_2 \rightarrow ArCONHOH + H_2O$$

AMINES

Amines are organic derivatives of ammonia in which one or more hydrogen are substituted by alkyl or aryl groups. Amines are classified as primary, secondary or tertiary depending upon the degree of substitution of nitrogen. In primary amines one alkyl or aryl group is attached to nitrogen. If two alkyl or aryl groups are attached to nitrogen, it is secondary amine.

Similarly a tertiary amine is obtained by replacement of all the three hydrogen atoms from a molecule of ammonia.

TEST TO DISTINGUISH BETWEEN PRIMARY, SECONDARY AND TERTIARY AMINES

EXPERIMENT	OBSERVATION	INFRENCE
1. **Nitrous acid test** (i) Blank test In a test tube take 5ml of dil.HCl , Cool in ice cold water and add 2ml of ice cold	There is evolution of N_2 gas.	

$NaNO_2$ solution . Observe. (ii)Test with amine Dissolve 0.2g of compound in 5ml of dil.HCl ,cool in ice cold water ,then add 2ml of ice cold $NaNO_2$ solution .Observe .	(i)The solution remains clear and there is much greater evolution of N_2 than in blank test. (ii) The solution remains clear and there is slightly greater evolution of nitrogen gas than in the blank test. (iii)A yellow oily layer is formed. (iv)A brown ppt. Is formed which changes to green on adding dil. NaOH solution.	Aliphatic primary amine group present Aromatic primary amino group present. Secondary amine present. Tertiary amine present.
2.**HINSBERG TEST** Take 0.5ml of given compound in a clear test tube, add 2ml of 25% NaOH, 2ml of water and 1ml of benzene sulphonyl chloride. Shake the contents for 10 minutes, cool under tap water, observe the formation of ppt. if ppt. is formed, add 2ml conc. HCl and observe.	(i) No ppt. Formed but some insoluble material is formed on addition of conc. HCl. (ii) ppt. Formed which does not dissolve in conc. HCl. (iii) ppt. Formed which dissolved in conc. HCl.	Primary amine present Secondary amine present Tertiary amine present

CHEMISTRY OF TESTS TO DISTINGUISH BETWEEN 1^0 , 2^0 , 3^0 AMINES

1. **NITROUS ACID TEST**

If the solution remains clear and there is much greater evolution of nitrogen gas compared to blank test, the primary aliphatic amine is indicated.

(i) $NaNO_3 + HCl \rightarrow HNO_2$

$R\text{-}NH_3 + HNO_3 + HCl \rightarrow R\text{-}N{\equiv}NCl + H_2O \rightarrow ROH + N_2 + H_2O + NaCl$

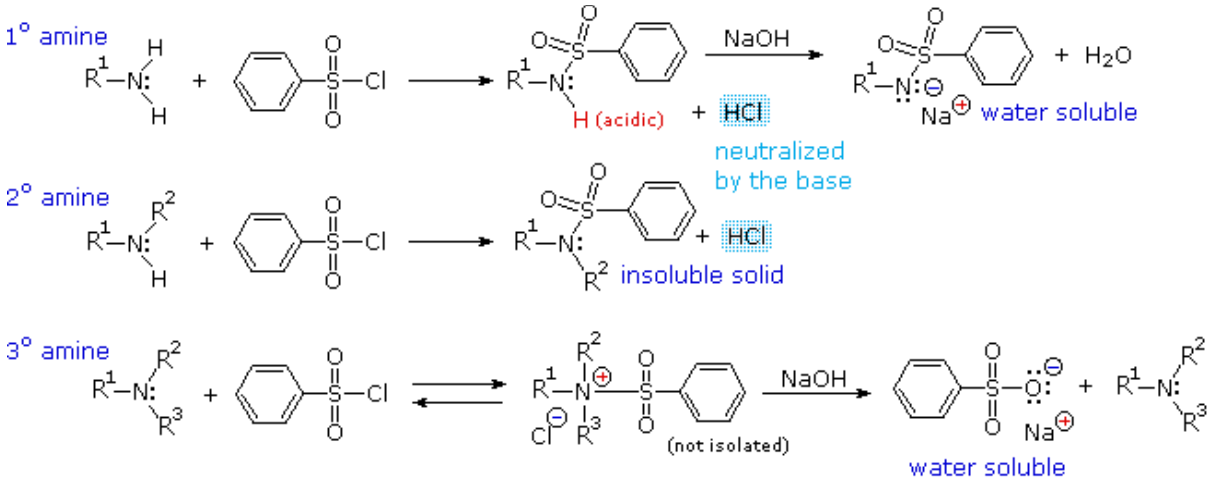
(ii) 2^0 amine

$^{R}_{R} > NH + HONO \rightarrow R_2N.NO + H_2O$

(II) If the solution remains clear but there is slightly greater evolution of N_2 than in the blank test, the primary aromatic amine is indicated.

2. **HINSBERG TEST**

A clear solution is obtained in NaOH ,which gives an insoluble matter on addition of conc. HCl indicates primary amine, precipitate is formed which is insoluble in NaOH indicates presence of secondary amine and tertiary amine do not react with Hinsberg reagent the precipitate formed are soluble in water.



PRIMARY AMINES (-NH_2 GROUP)

IDENTIFICATION TESTS FOR PRIMARY AMINE (-NH_2 GROUP)

EXPERIMENT	OBSERVATION	INFRENCE
1.**NITROUS ACID** TEST:Dissolve about 0.2g of	Brisk effervescence of	Primary aliphatic or aromatic

compound in 5.0 ml if dil.HCl, cool in ice cold water, then add 2ml of ice cold $NaNO_2$ solution.	nitrogen gas	amine present.
2.**CARBYLAMINE TEST** Take 0.2g of solid KOH in a dry test tube, add 2-3 ml of ethanol, warm the contents until KOH dissolves. To this add few drops of chloroform and a pinch of given compound.	Offensive smell	Primary aliphatic or aromatic amine present

CHEMISTRY OF THE TEST FOR PRIMARY AMINE

1. **NITROUS ACID TEST**:

$$R\text{-}NH_2 + NaNO_2 + HCl \rightarrow ROH + N_2 + H_2O + NaCl$$

2. **CARBYLAMINE TEST**:

$$R\text{-}NH_2 + CHCl_3 + 3KOH\ (alc.) \rightarrow RNC + 3KCl + H_2O$$

DISTINGUISING TESTS BETWEEN ALIPHATIC AND AROMATIC PRIMARY AMINES

EXPERIMENT	R-NH_2(aliphatic amine)	Ar-NH_2
1. **Rimin's test**: To a pinch of organic compound in the test tube add 5.0 ml of water ,shake then add 1.0ml of acetone and 2-3 drops of sodium nitroprusside solution.	Violet colour appears within two minutes.	No reaction
2. **Azo dye test**:		

Take a pinch of organic compound add 5ml of dil. HCl and cool in ice to 0-5^{0}C. Add 2ml of ice cold $NaNO_2$ solution. Shake the contents .in another test tube, dissolve about 1.0g of β-naphthol in NaOH solution. Cool to 0-5^{0}C and mix it with the first solution.	No reaction	A brilliant orange or red dye is formed.
3. **Bleaching powder test**: Shake a small amount of organic compound with 5.0ml of water, then add a few drops of bleaching powder solution.	No reaction	Purple colouration which soon turns brown.

IDENTIFICATION TESTS FOR SECONDARY AMINES

EXPERIMENT	OBSERVATION	INFRENCE
1. **Nitrous acid test**: Dissolve 0.5g of organic compound in 5ml of dil. HCl .in another test tube, dissolve about 1.0g of $NaNO_2$ in 5.0ml of water. Cool both the solutions in ice bath. Mix the two solutions.	A yellow oily layer separates at the bottom.	Secondary amine present.
2. **Simon's test**: To the solution of organic	Deep blue or violet colour	Secondary amine present.

compound in ethyl alcohol, add few drops of sodium nitroprusside solution. Then add few drops of dil. acetaldehyde solution.	formed.	

CHEMISTRY OF NITROUS ACID TEST:

$$R_2NH + HONO \rightarrow R_2N.NO + H_2O$$

IDENTIFICATION TEST FOR TERTAIRY AMINE

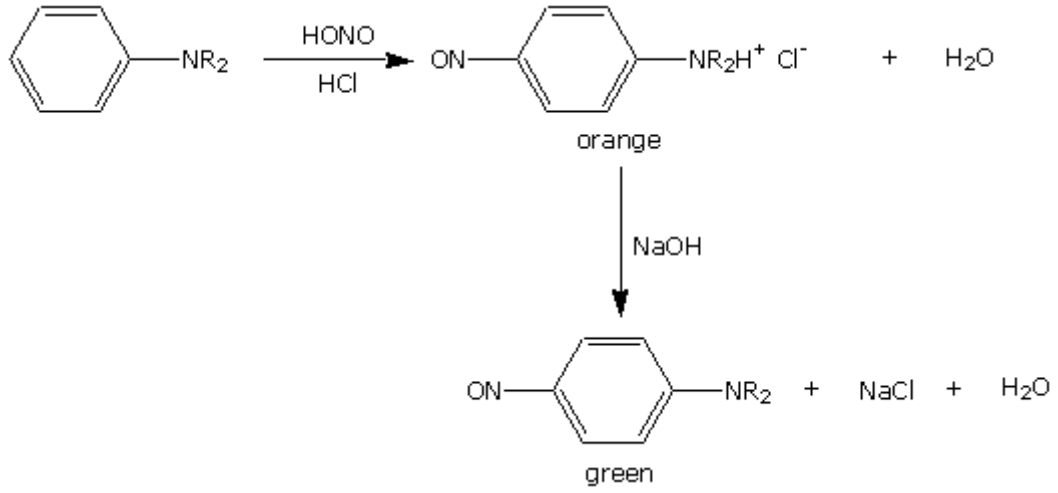
EXPERIMENT	OBSERVATION	INFRENCE
METHIODIDE FORMATION: To a small amount of compound ,add 4-5 drops of methyl alcohol and 2-3 drops od methyl iodide. The mixture becomes hot. Cool the contents.	Colourless crystals formed	Tertiary amine present
NITROUS ACID TEST: Dissolve a pinch of organic compound in dil. HCl in another test tube dissolve 1.0g of $NaNO_2$ in 5.0ml of water. Cool the solution in bath and mix the two solutions.	Brown ppt is obtained which turns bright green on adding few drops of NaOH.	Tertiary amine present.

CHEMISTRY OF TESTS FOR TERTAIRY AMINES

1. **Methiodide formation test**:

$$R_3N + CH_3I \rightarrow R_3N^+CH_3I^-$$

2. **Nitrous acid test**: Nitrous acid does not react with aliphatic tertiary amines nor does it react with aromatic tertiary amine which contain three aryl groups like $(C_6H_5)_3$ N.



4) Titrate given solutions of HCI and NaOH potentiometrically.

Requirements: Potentiometer, battery, standard cell, calomel electrode, platinum electrode, quinhydrone, beaker, stirrer, burette, N/10 HCI, N/10 NaOH.

Theory

This is an equilibrium mixture of quinone and hydroquinone. This cannot be used in strongly base solution (pH>9) because the equilibrium will be disturbed. Quinone - hydroquinone system involves the equilibrium

$C_6H_4O_2 + 2H+ + 2e \rightleftharpoons C_6H_6O_2$

(Quinone) (hydroquinone)

Q QH_2

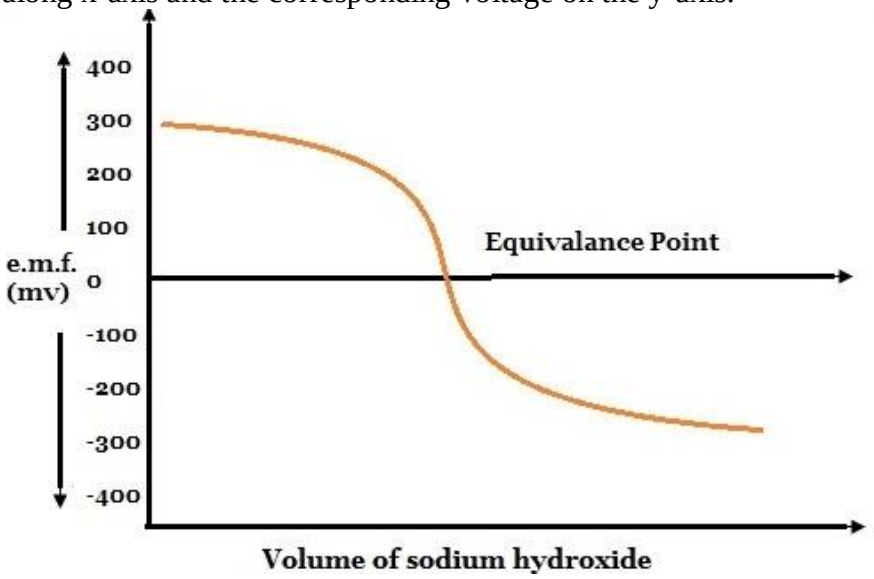
For this reduction reaction, the potential developed on the platinum electrode immersed in this system is given by Nernst equation

$$E_{el} = E^0_{el} - [-\frac{2.303RT}{2F}\log\frac{[Q]}{[QH_2]} - \frac{2.303RT}{F}\log H^+]$$

$$E_{el} = E^0_{el} + \frac{2.303RT}{2F}\log\frac{[Q]}{[QH_2]} + \frac{2.303RT}{F}\log H^+]$$

instead of taking quinone & hydroquinone a small amount of quinhydrone, which is an equimolar compound of quinone Q and hydroquinone QH2 is taken. QH2 is a weak acid; its ionisation is very small particularly if the pH of the solution is less than 7. Therefore, the concentration of hydroquinone, QH2 is same as that of quinone Q i.e. [Q]/[QH2] = unity and hence the Nernst equation may be written as, = Eo + 0.0591 log [H+] at 25 o C = Eo – 0.0591 pH The standard electrode potential of quinhydrone electrode Eo = + 0.6996 V E = 0.6996 – 0.0591 pH Thus the potential of quinhydrone electrode is dependent on pH of the solution i.e. quinhydrone electrode behaves as a reversible hydrogen electrode. This is less expensive than hydrogen electrode since it can be set up easily simply by adding a pinch of quinhydrone to the solution and inserting a Pt electrode for making electrode connections. The basic reaction involved in the neutralisation of an acid with a base is H+ (aq) + OH^-(aq) ⇌ H2O ...(1) Therefore, the indicator electrode to be selected is obviously an electrode reversible to H+ ions. e.g. Hydrogen electrode, glass electrode or quinhydrone electrode. For better results and simple experimentation the latter electrode is commonly used. The cell setup using quinhydrone electrode is: SCE | quinhydrone (acid solution) | pt ... (2) A known volume of the acid is taken in the beaker.

Standard NaOH is taken in the burette and is added in small volume each time. The value of potential is noted after each addition. A graph is plotted by taking the volume of NaOH added along x-axis and the corresponding voltage on the y-axis.



End point in an acid- alkali titration. Zero EMF point and inflexion point on the curve may or may not coincide.

Procedure: Take 20 ml of the acid solution (or any other measured volume enough to allow the indicator electrode and reference electrode to dip into the solution) in a beaker.Add a pinch of quinhydrone to saturate the solution. Dip the indicator (Pt) and reference (SCE) electrodes in the solution . If a dip type SCE is not available, the beaker solution can be put in contact with a laboratory type SCE through a salt bridge. Connect the two electrode leads to a potentiometer. If we find that the cell e.m.f. is not in the range of the potentiometer, the terminal connections at the potentiometer may be interchanged to obtain appropriate contact of +ve and -ve poles of cell to the potentiometer +ve and -ve poles. Record the initial value of cell e.m.f.

Add the alkali solution in 1 ml lots. Shake well after each addition and measure the cell EMF at each stage. From this rough titration find out the approximate volume needed for reaching the end point.
Repeat the titration by adding 1 or 2 ml of alkali in initial steps but by adding one or two drops of alkali at a time in the ml step expected to involve the end point. Subsequent additions can be made in steps of 1 or 2 ml of alkali again.
Plot a graph between cell EMF (Y-axis) and volume of alkali used (X-axis). From this graph read the end point volume of the titrant .

It may be pointed out that so long as quinhydrone is to be used as an indicator electrode, we shall have to take the acid solutions in the beaker and alkali in the burette. It is so because in alkaline solutions the hydroquinone part of quinhydrone is rapidly oxidised by aerial oxygen.

Cell used : SCE | Acid solution | Pt
|Quinhydrone|

Volume of acid solution taken =
Normality of alkali used =

S.No	Volume of alkali used	E.M.F of cell

Calculations:

$N_1V_1 = N_2V_2$

Where

N_1 is the strength of acid (known)

N_2=?

V_1 is the volume of acid taken in beaker (known)

V_2=Volume of alkali from graph