SEPARATION OF MIXTURES

1- General Discussion:

To identify the component of mixture, the general procedures adopted involve the separation of the mixture into individual components and identification of each component thus separated. The separation of a mixture is tedious process and requires a lot of skill and genius. In fact, successful separation of a mixture depends upon the nature of its components. And much upon your general and experimental knowledge of organic chemistry. No definite scheme can be complied to cover all the possible way of separating mixtures, rather an outline of the methods employed can be given. You should understand that the separation should be carried out in such manner that each compound is obtained in a pure state. It renders the identification of individual compounds much easier.

1.1. PRELIMINARY EXAMINATION OF A MIXTURE

A preliminary examination of a mixture makes separation quite easy. For this propose, frist of all you should find out the elements present in the mixture, and then depending upon the various elements present therein, the tests of various group should be done. This will enable you to get a rough idea about the nature of the components present in the mixture.

Various combination of neutral, acidic and basic substance are generally given in the form of mixture:

1-Liquid (miscible or immiscible)

2- Solid and liquid (suspension or solution)

3- Solids

A mixture of two immiscible liquids can be separated with the help of a separation funnel and a suspension of a solid in the liquid by filtration.

Separation of the components of a homogeneous mixture (liquid or solid) can be effected by taking advantage of any one or more of the following properties:

I.1. difference in the volatility of the components.

I.2. Difference in the solubility of the components.

I.3. Difference in the chemical behavior of the components.

I.1. SEPARATION BASED ON THE DIFFERENCE IN VOLATILITY OF THE COMPONENTS

A mixture of miscible neutral liquids can be separated by fractional distillation if the boiling point of the various components present differ significantly (e.g. a mixture of carbon tetrachloride (b.p. 77) and toluene (b.p. 111). Steam-volatile component can be separated from the mixture by steam distillation. Solutions of solids in liquids can be separated by distilling off the liquid and collecting the solid in the form of residual.

I.2. SEPARATION BASED ON THE DIFFERENCE IN SOLUBILITY OF THE COMPONENTS

Separation of mixtures through this technique is based on the principle of “like dissolves like”, this means that a polar solvent may be expected to readily dissolve only polar solute and a nonpolar solvents only nonpolar solutes. The introduction of several polar functional groups into a molecule decreases its solubility in nonpolar solvents, such as ether, carbon tetrachloride and benzene. Thus, a mixture can be separated with the help of water or organic solvents.

The simplest procedure is to try these solvents one after another starting from water, every time a small quantity of the mixture is shaking with the solvent used. If one component seems to have been dissolved either when the mixture was cold or on being warmed in that particular solvent, then it is filtered. The residue is washed with a little more of the same solvent and dried to get the insoluble component, whereas the second component is obtained by simply evaporating the solvent on a water bath.

I.3. SEPARATION BASED ON THE DIFFERENCE IN CHEMICAL BEHAVIOR OF THE COMPONENTS

In this method, the effect of the mixture on moist blue and red litmus paper is observed. For instance, if blue litmus turn red, one can draw the inference that at least one of the components is either an acidic or phenol. If the red litmus turn blue it show that the components is basic in nature and may be amine, etc. the acidic component can be conveniently separated by shaking the mixture with saturated solution of sodium bicarbonate; phenol with dilute sodium hydroxide solution; and the basic component with diluted hydrochloric acid. These separations should preferably be tried only when the components are insoluble in water and have failed to separate by water.

1.3.1. SEPARATION BY SATURATED SODIUM BICARBONATE SOLUTION

The mixture is shaken will with a saturated solution of sodium bicarbonate and filtered. The residue is shaken again and again with a fresh quantity of sodium bicarbonate solution until it is free from the soluble component, finally washes with water and dried. To the filtrate in a beaker, add dilute hydrochloric acid in a small portion, all the while stirring the solution until precipitation is complete. The precipitate is filtered, washed well with water and dried. Weakly acidic compounds (e.g. phenol) cannot be separated by this method; only mixtures of acidic compounds differing in acidity can be separated.

I.3.2. SEPARATION BY 2N-SODIUM HYDROXIDE SOLUTION

Both carboxylic acid and phenol dissolve in sodium hydroxide solution and hence can be separated from the other components in the mixture. However, it is desirable to separate carboxylic acids by sodium bicarbonate solution and to limit this method to the separation of phenolic compounds only. The process of separation remains the same as that described under sodium bicarbonate solution. In case, on acidification, a cloudy solution is obtained and no solid precipitate is formed, then extract the sparingly soluble component (phenol) twice with equal amounts of ether in a separatory funnel. The ethereal layer is separated and the ether is distilled off to get the residue (phenol).

I.3.3. SEPARATION BY 2N-HYDROCHLORIC ACID

The mixture, containing a base as one of the components, is shaken well with 2N hydrochloric acid repeatedly until it is free from the soluble fraction. The base is precipitated from the filtrate by the addition of dilute sodium hydroxide solution in small portions until precipitation is complete, all the while stirring the mixture. The separate is filtered out, washed well with water and dried. If an oil or oily suspension is obtained, it is extracted in the same manner with ether as sodium hydroxide solution.

I.3.4. MISCELLANEOUS METHODS OF SEPARATION

I.3.4.1.Separation by sodium bisulphite

The mixture, containing a carbonyl compound (aldehyde and ketone) as one of the components, can be separated by shaking it with an aqueous solution of sodium bisulphite. This converts the carbonyl compound into a water-soluble bisulphite derivative which after separation is decomposed by familiar methods to get the pure component.

I.3.4.2. Separation by acetyl or benzoyl chloride

Primary and tertiary amines can be separated by subjecting the mixture to acetylation or benzoylation reactions. This converts the primary amine to a neutral amide. The tertiary amine ,then is removed by extracting the mixture with diluted hydrochloric acid. Primary amine(which is left behind in the form of an amide) can be recovered from the amide by hydrolysis.

I.3.4.3.Separation by fuming sulphuric acid

Aromatic hydrocarbons are frequently, separated from paraffinic or alicyclic hydrocarbons by fuming sulphuric acid. This converts the aromatic hydrocarbon into highly polar sulphonic acids which can be separated and reconverted to the hydrocarbon by treatment with super heated steam.

I.3.4.4.-Separation by specialized techniques

A mixture can be separated by certain specialized methods, such as paper chromatography, column chromatography, vapor phase chromatography, etc.

II. Carboxylic acids

Organic acids can be classified into aliphatic acids and aromatic acids:

<u>II.1. Aliphatic acids</u>

All aliphatic acids such as (oxalic, tarataric, maleic, fumaric, succinic, malonic, citric and formic acids) are readily soluble in water, except succinic and fumaric acid, which are sparingly soluble in cold water.

<u>II.2.Aromatic acid</u>

Aromatic acids such as benzoic, phthalic, cinnamic, phenylacetic and salicylic acids are insoluble in water. Phenyl acetic, salicylic and phthalic acids are moderately soluble.

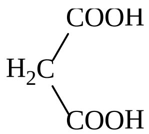
All acids dissolve in alkali and carbonate solution with evolution of CO_2 *<u>(distinction from phenol)</u>*, also readily dissolve in NaOH solution.

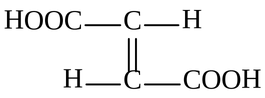
$$RCOOH + Na_2CO_3 \longrightarrow RCOONa + H_2O + CO_2$$

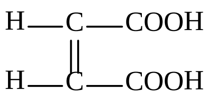
$$RCOOH + NaOH \longrightarrow RCOONa + H_2O$$

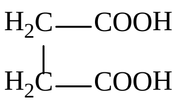
II.3. Identification of acids:

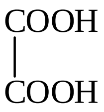
<u>II.3.1. For aliphatic acids:</u>

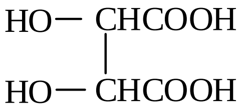

Malonic acid

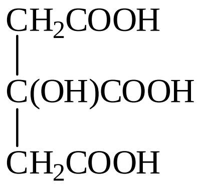

Fumaric acid


Maleic acid


Succinic acid


Oxalic acid


Tartaric acid


Citric acid

II.3.1.1- Neutral solution of an acid:

Add ammonia solution in excess to a small amount of the solid acid or its solution heat until no more ammonia gas evolved. The residual solution is the neutral solution.

$$RCOOH \quad + \quad NH_4OH \longrightarrow RCOONH_4 \quad + \quad H_2O$$

II.3.1.2. Effect of heat on solid:

Odour of acetic acid, (Solution of solid in acetic anhydride →yellowish red solution with green fluorescence) Malonic acid

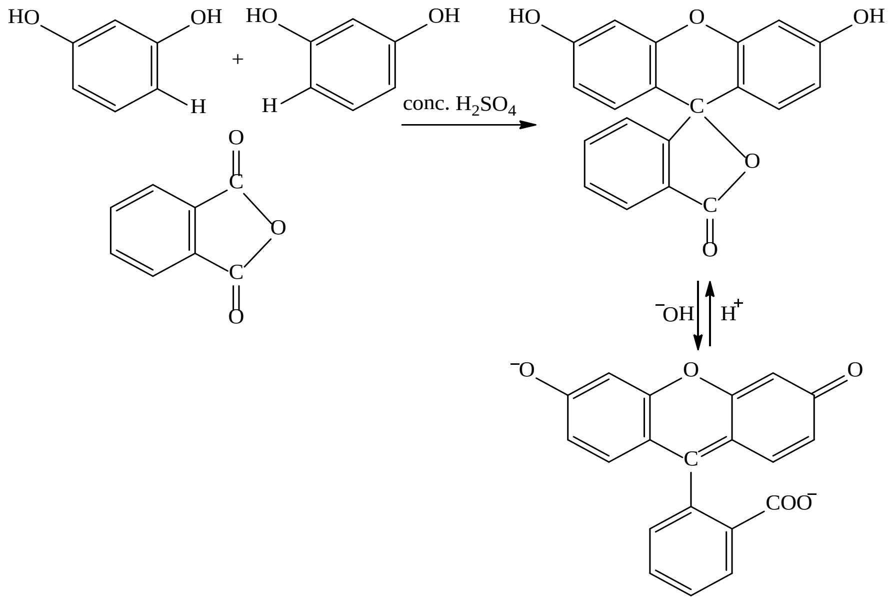
$$CH_2(COOH)_2 \xrightarrow{\triangle} CH_3COOH \quad + \quad CO_2$$

II.3.1.3. Neutral solution + neutral $FeCl_3$:

Observation	Inference
Red colour turn brown ppt. by boiling.	Maleic acid (unsaturation test +ve , fluorescein test, red colour).
Red brown ppt.	Fumaric(unsaturation test +ve , fluorescein test, red colour).
Buff ppt.	Succinic acid (fluorescein test green)
No specific result (-Ve)	Oxalic , tartaric or citric acid

II.3.1.4.Fluorescein test:

In a clean dry test tube, fuse carefully, few crystals of resorcinol and an equal quantity of the solid acid, or its anhydride moistened with 2 drops of conc. H_2SO_4 and then pour into excess NaOH.



II.3.1.5. Neutral solution + $CaCl_2$:

Observation	Inference
White ppt. immediately on cold insoluble in acetic acid.	Oxalic acid
White ppt. after scratching and warming, soluble in acetic acid.	Tartaric acid (Fenton's test)
White ppt. after boiling, insoluble in acetic acid	Citric acid (Deng's test)

Deng's test:

Add 1 ml of Deng's No. 1 to 3 ml of neutral citric acid and heat to boiling then add two drops of Deng's No. 2 and note that the colour of pot. permanganate is discharged. (redox reaction).

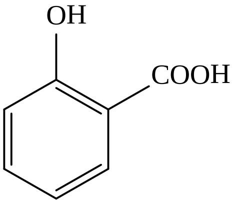
Fenton's test:

Add one drop of $FeSO_4$ soln. to 2 ml of tartaric acid soln, then add drop wise H_2O_2 soln. till the solution acquires a green colouration. Add excess of NaOH soln. a violet colour is produced. (redox reaction).

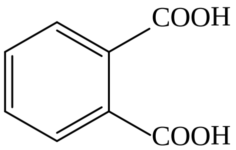
II.3.1.6. Unsaturation test:

A solution of acid in sod. Carbonate, discharge the colour of bromine water and also a dilute $KMnO_4$ solution.

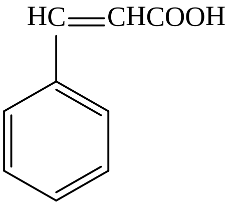
II.3.2. For Aromatic Acids:



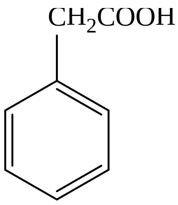
Salicylic acid



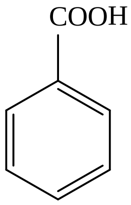
Phthalic acid



Cinnamic acid



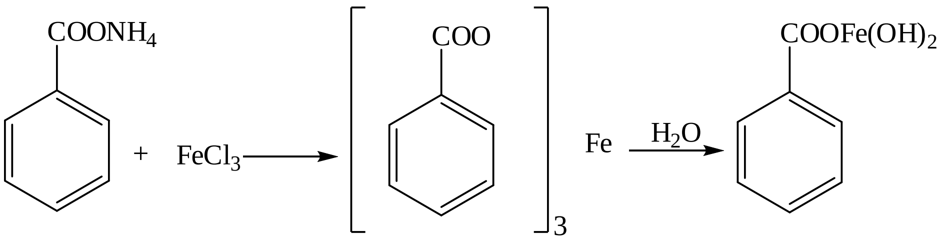
Phenylacetic acid



Benzoic acid

II.3.2.1.Neutral solution of acid + neutral $FeCl_3$:

Observation	Inference
Violet colour.	Salicylic acid
Buff ppt.	Phthalic , Cinnamic, Phenylacetic or Benzoic acid



Amm. Benzoate

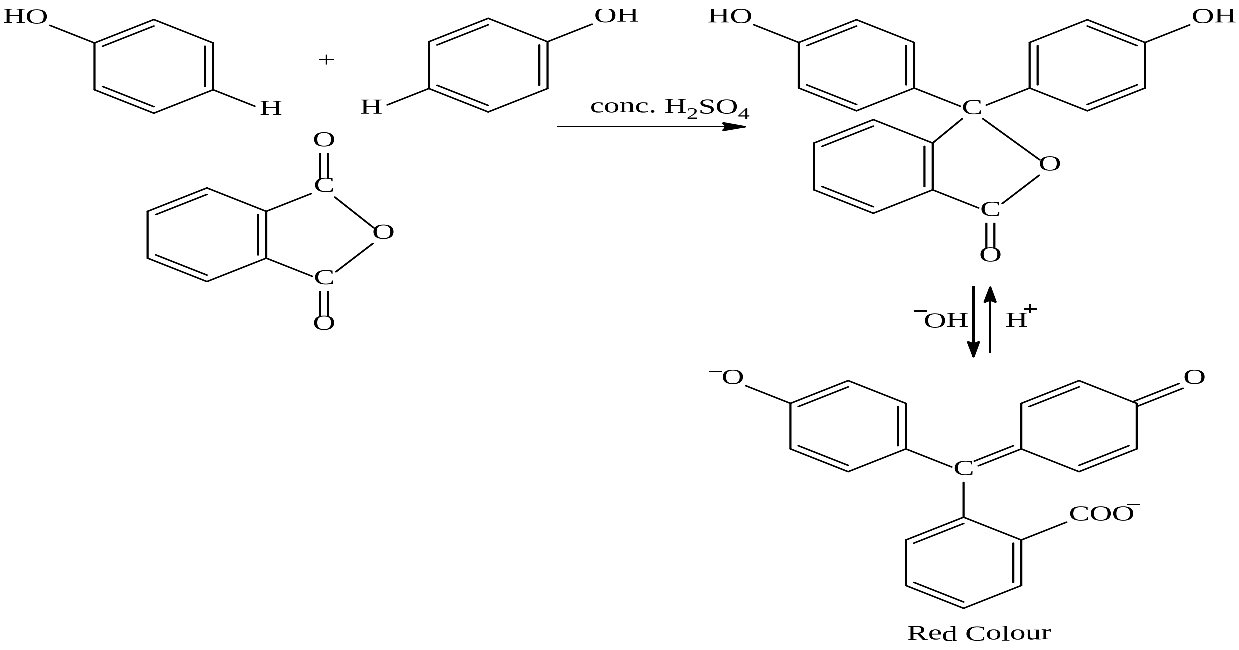
Ferric benzoate

Basic Ferric benzoate
(Buff ppt)

To differentiate between phthalic and benzoic acic carry out the following experiments:

II.3.2.1.1. Phthalein test:

In a clean dry test tube, fuse carefully a few crystals of the acid and two drops of phenol, moistened with two drops of conc. H_2SO_4. Pour into excess NaOH, if red colour, then phthalic acid.

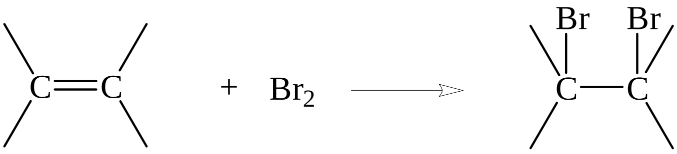


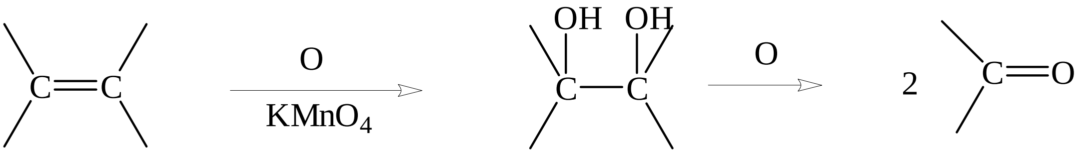
II.3.2.1.2. Fluorescein test:

+ve phthalic acid

II.3.2.2. Unsaturation test:

If the solution of the substance is sod.carbonate discharge the colour of bromine water and also a few drops of dilute $KMnO_4$, then cinnammic acid .





II.3.2.3. Boiling test:

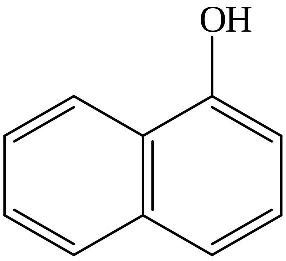
Boil the acid in water, if oily drops are formed, then phenyl acetic acid.If all the above tests (phthalein, fluorescein, unsaturation and boiling with water) are negative, then Benzoic acid.

III. Phenols

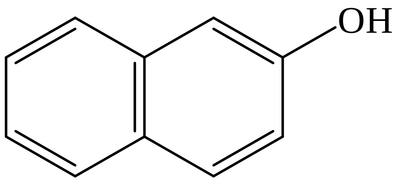
Phenols can be classified into water soluble and water insoluble :

III.1. Water insoluble phenols

Water-insoluble phenols such as: α-naphthol and β-naphthol.



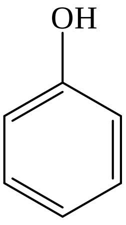
α -Naphthol



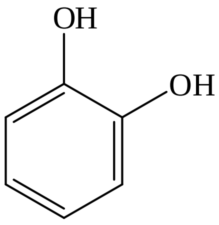
β-Naphthol

III.2. Water soluble phenols

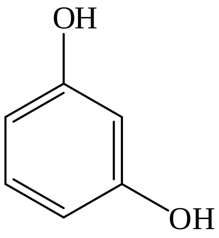
Water-soluble phenols such as: Catechol, resorcinol, quinol, pyrogallol and phenol.



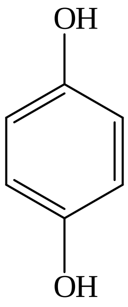
phenol



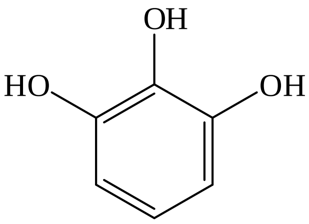
catechol



resorcinol



hydroquinone



pyrogallol

Phenols distinguish themselves from the carboxylic acids by the insolubility in alkali carbonate solution (Na_2CO_3).

They form soluble sodium salts with NaOH, so insoluble phenols are soluble in NaOH solution and reprecipitated by the addition of conc. HCl.

$$ROH + NaOH \rightarrow RONa + H_2O$$

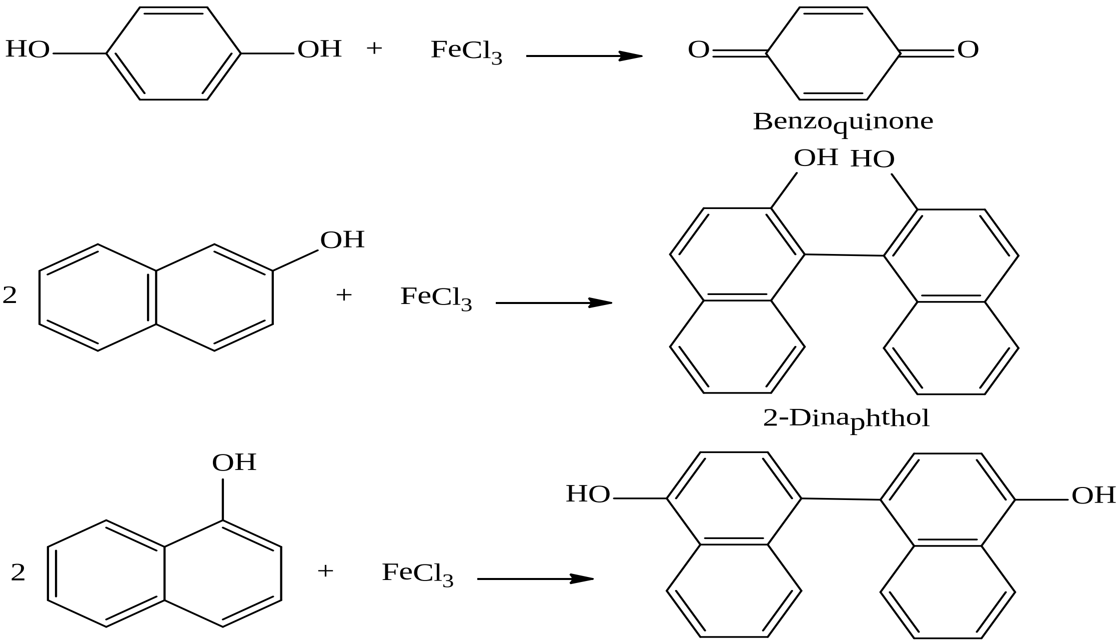
phenol	m.p.°C	Properties	Specific test
Phenol	42	Colourless or pinkish-brown, carbolic odour, sparingly sol. in H_2O, sol. in hot H_2O, alc.	• Phthaline test→red colour • Comp. + Zn dust ⟶ benzene
Catechol	105	Colourless, faint carbolic odour, sol. in H_2O, benzene and ether	• Comp. + Fehling solution + heat ⟶ red ppt.
Resorcinol	113	Colourless or pinkish, sol. in H_2O, alc., ether	• Denig's test (+ve) • Comp. + aq. NaOH + $CHCl_3$ + heat→red or violet- red colour
Quinol	170	Colourless, sol. in H_2O, alc., ether and insol. in benzene	• Comp. + H_2O + $FeCl_3$→blue colour • Comp. + amm. $AgNO_3$⟶ grey or black ppt.
Pyrogallol	133	Colourless or black, sol. in H_2O, alc., ether	• Comp. + H_2O + $FeSO_4$⟶ blue-violet colour or ppt.
α-Naphthol	94	Colourless or violet, faint odour, sparingly sol. in H_2O, sol. in alc. and ether	• Comp. + aq. NaOH + CCl_4 (one drop) + Cu (powder) + heat⟶blue colour
β-Naphthol	123	Colourless or pinkish-brown, faint odour, insol. in H_2O, sparingly sol. in hot H_2O, sol. in alc., ether and benzene	• Comp. + KOH + $CHCl_3$ (50 °C)⟶blue colour

III.3.3. Identification of phenols

III.3.3.1. $FeCl_3$ Test:

Hydroxyl group of phenols and enolic compounds gives characteristic colour (usually violet) with $FeCl_3$. This test is valid in presence of carboxylic group with phenolic group.

Add few drops of neutral $FeCl_3$ solution to a solution of the phenolic substance in water (or in alcohol) and then, note the colour produced.



Aq. or alc. soln. of phenol + Neutral $FeCl_3$

Observation	Inference
Violet color discharged by HCl.	Phenol
Violet color discharged by AcONa.	Resorcinol
Green color turns red by NaOH.	Catechol
Green color crystals	Hydroquinone
Red-turns violet color by NaOH.	Pyrogallol.
Greenish turns to violet	α-Naphthol.
Faint green color	β-Naphthol.

III.3.3.2. Liebermann-nitroso reaction:

Phenol reacts with nitrous acid to give p-nitrosophenol which condense with excess phenol to give indophenols (usually red). When solution of the latter is rendered alkaline, a blue or green anion is liberated.

To a small amount of $NaNO_2$ in a clean dry test tube, add 0.5g of phenol and heat gently for 1 min., cool, add 1 ml of conc. H_2SO_4 with shaking. A deep green or blue colouration develops (sometimes only after 1-2 min.). Dilute cautiously, the solution, turns red. Now add an excess of NaOH soln., the green or blue colouration reappears.

Phenol	H_2SO_4	Dilution	NaOH
phenol	Green or blue	Red	Green or blue
α-Naphthol.	Green		
β-Naphthol.	Brownish back		
Catechol	Deep green	Dirty brown	Red
Resorcinol	Deep blue	Red	brown

II.3.3.3. Chloroform and NaOH (Reimer-Timann reaction):

Dissolve the phenol in conc. NaOH (20%), add 1-2 ml of chloroform and heat gently. Notice the colouration produced.

Phenolic comp.	colour	Phenolic comp.	colour
phenol	No-colour	Catechol	Green
α-Naphthol.	Deep blue fading to green	Resorcinol	Red with fluorecence
β-Naphthol	Deep blue fading to green	Quinol	-ve

IV- ORGANIC BASES

Organic bases may be primary amines (*p*-toluidine, *α*-naphthylamine *β*–naphthylamine or nitro anilines (*o*-, *m*-, or *p*-), and secondary amines (diphenylamine).

All amines are very sparingly soluble in water, but dissolve readily in dilute mineral acids (except naphthylamine,which are only moderately soluble in acids) and reprecipitated by NaOH solution.

The presence of nitro group decreases the basicity of the amino group and thus decreases the solubility in acids but increases their solubility in water.

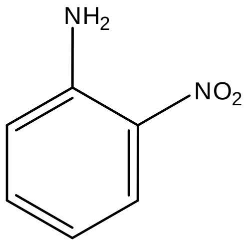
Amine	M.P.(^{o}C)	Properties	Specific test
Aniline	B.P. 183	Colourless or reddish, oily, characteristic odour, slightly sol. in H_2O, sol in alc, ether, benzene.	Azodye test → +ve
p-toluidine	44	Colourless or grey, oily, characteristic odour, almost insol. in H_2O, sol in alc, ether.	Azodye test → +ve
α – Naphthylamine	50	Colourless , insol. in H_2O, slightly sol. in hot H_2O, sol in alc., ether.	*Azodye test → +ve *Compd.+ dil. HCl + $FeCl_3$ → Blue ppt.
B - Naphthylamine	112	Colourless , pinkish, sol. in hot H_2O, sol in alc., ether.	Azodye test → +ve
***o* - Nitroaniline**	71	Orange yellow, slightly sol. in hot H_2O and sol in alc.or ether.	Azodye test → +ve

***m*-Nitroaniline**	114	Yellow, insol. in H_2O, less sol in alc., ether, and sol. in hot H_2O.	Azodye test → +ve
***p*-Nitroaniline**	147	Yellow, insol. in H_2O,and less sol in alc., ether, sol. in hot H_2O.	Azodye test → +ve
Diphenylamine **$(C_6H_5)_2NH$**	54	Colourless , insol. in H_2O, and sol in alc. or ether	Compd. + conc. H_2SO_4 or HCl + 2.5% aq. $NaNO_2$ → deep blue colour

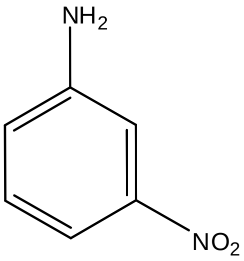
IV.1. Identification of organic bases:

IV.1.1. Differentiation between nitro anilines

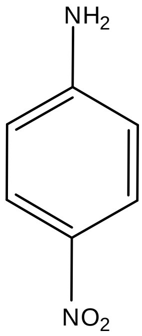
All nitro anilines are yellow or orange coloured compounds and are soluble in hot water.



o-Nitroaniline

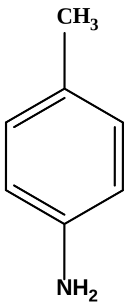


m-Nitroaniline

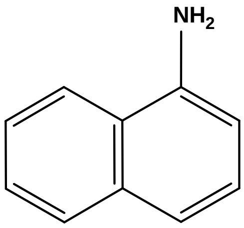


p-Nitroaniline

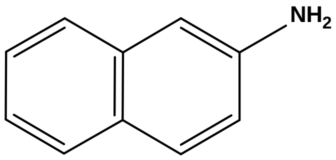
IV.1.2. Differentiation between primary amines.



P-toluidine



α-naphthylamine



β-naphthylamine

IV.1.2.1. Boiling test

Boil the solid substance in water, and observe if oily drops are formed:

Observation	Inference
No oily drops	*β*-naphthylamine
Oily drops	*P*-toluidine or *α*-naphthylamine

IV.1.2.2. Alcoholic solution of primary amine + $FeCl_3$

Observation	Inference
Blue colour	*α*-naphthylamine
-ve	*P*-toluidine

IV.1.3. Confirmatory test:

IV.1.3.1. formyl derivatives:

Formic acid condenses with primary and secondary amines to yield formyl derivatives:

$$\mathrm{ArNHR} \quad + \quad \mathrm{HCOOH} \quad \xrightarrow{\qquad\qquad} \quad \mathrm{ArN(CHO)R} \; + \; H_2O$$

Reflux 0.5 g. of the amine with 5 ml. of 90% formic acid (caution in handling) for 10 minutes, and dilute the hot solution with 10 ml. of cold H_2O. Cool in ice and, in some cases, saturate with salt if the derivative does not separate immediately. Filter, wash with cold H_2O and recrystallize from water, ethanol or light petroleum.

P-toluidine (*N*-formyl)	m.p. 53°C
α-naphthylamine (*N*-formyl)	m.p. 139°C
β-naphthylamine (*N*-Formyl)	m.p. 129°C

IV.1.3.2. Action of bleaching powder (oxidation)

Shake 1-2 drops of amine with 10 ml. of H_2O and add a few drops of bleaching powder soln. (Sodium hypochlorite ,NaOCl), characteristic colour appears.

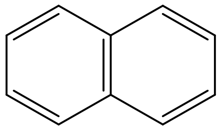
Observation	Inference
Yellowish colour	*P*-toluidine
Pale brown	α-naphthylamine
-ve	β-naphthylamine

V. Neutrals

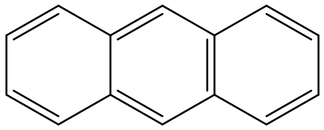
Neurals could be classified into hydrocarbon, carbohydrates and salts of acid.

V.1. Insoluble neutrals (hydrocarbons)

They contain the benzene nucleus in its molecules e.g. naphthalene and anthracene.



Naphthalene



Anthracene

Hydrocarbon	B.P.	Properaties	Specific test
Naphthalene	80	Colourless, unpleasant penetating odour, insol. In H_2O, sol. In hot alc, ether, benzene	• Compd. + $KMnO_4$ (solid)+ concH_2SO_4+resorcinol+heat and pour into NaOH → green fluorescence.
Anthracene	216	Colourless or pale yellow, insol. In H_2O, sol. In benzene	• Picrate test → red colour

V.1.1. Identification of hydrocarbons

Many hydrocarbon form molecular compound with picric acid, for example, naphthalene picrate $C_{10}H_8$-$C_6H_2(NO_2)_3OH$.

V.1.1.1. Picrate test:

Heat 0.1 g of the hydrocarbon and 0.2 g of picric acid separately in the minimum volume of acetone (about 2 ml), mix the two solutions and allow to cool. Filter and wash with 2 ml of ethanol.

Observation	Inference
Yellow crystals	Naphthalene (150 ºC)
Red crystals	Anthracene (138 ºC)

V.1.1.2. Nitation test:

Nitric acid reacts with naphthalene, in the presence of glacial acetic acid to give a nitronaphthalene while anthracene is not readily nitrated with nitric acid.

Dissolve by heating, 0.5 g of naphthalene in 3 ml of glacial acetic acid, then cool and add 1ml of conc. HNO_3 and heat the mixture gently for 1 minute. Cool and pour the solution into a beaker which contains about 25 ml of cold H_2O and note the separation of nitronaphthalene as a yellow solid.

V.2. Carbohydrates

They are classified into: **Monosaccharides** (glucose, galactose and fructose), **Disaccharides** (sucrose, maltose, and lactose) and **Polysaccharides** (starch and insulin). Mono and Disaccharides are soluble in water. They are detected by Molisch's test.

V.2.1. Molish test:

1 ml of carbohydrates soln + 1 ml of α-naphthol + 1-2 ml conc. H_2SO_4 down the slide of the test tube, a violet ring spread on shaking is formed. If the violet color disappears on shaking, the test is –ve and no carbohydrates is present.

V.2.2. Identification of Carbohydrates:

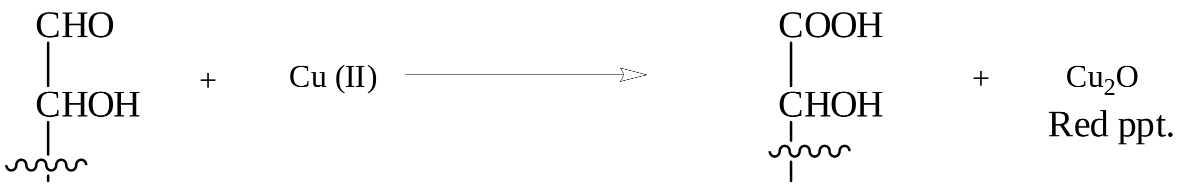
V.2.2.1. Iodine test:

To 2 ml of colloidal starch in water add one drop of iodine solution, if a blue color is developed, then we have Starch.

V.2.2,2. Barfoed’s Test

Heat a test-tube containing 1ml of the Barfoed's reagent and 1ml of a dilute solution of the carbohydrates in a boiling water bath.

Observation	Inference
Red Cu_2O ppt. within 2 minutes	Monosaccharides
Red ppt. after 5 minutes boiling No red ppt.	Disaccharides polysaccharide



Glucose Gluconic acid

V.2.2.3. Differentiation between Monosaccharides (Glucose or Fructose):

1.Rapid furfural:

1 ml of sugar soln. + 1 ml of α-naphthol, then add 5 ml of conc. HCl, boil, immediate violet color appears, then we have fructose.

Note: If the violet color appears after boiling for 1-2 min., then we have Glucose.

2. Furfural test:

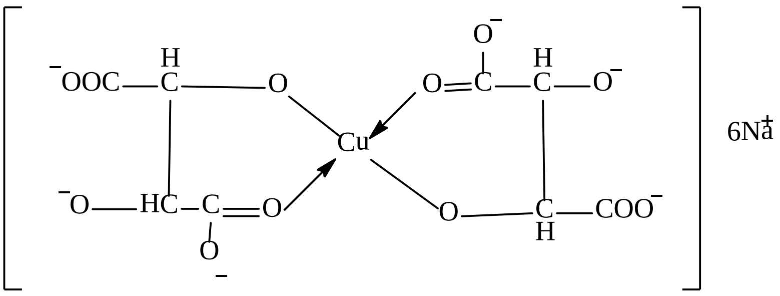
0.2 g of carbohydrates + 6 ml dil. HCl, boil, then insert an aniline acetate paper at the mouth of the test tube, continue boiling, the paper acquires a red color in case of Fructose.

V.2.2.4. Differentiation between Disaccharides (Maltose, Sucrose and Lactose)

1-Fehling's solution:

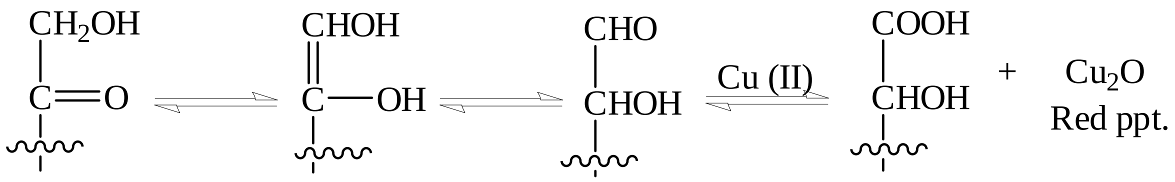
(**Fehling 1** : $CuSO_4$. H_2O and **Fehling 2** : sod., pot. Tartarate in NaOH soln.)

Place 3 ml of Fehling's solution(prepared by mixing equal volumes of Fehling's solution of 0.1g of the carbohydrate in 2 ml of H_2O and continue the boiling for 1-2 minutes.



Fehling reagent

Observation	Inference
A yellow-red Cu_2O ppt.	Reducing sugar (Maltose or Lactose)
No red ppt.	Non reducing sugar (sucrose)

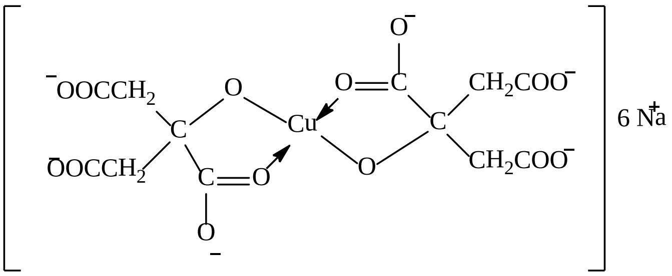


Ketose **Enediol** **Aldose**

2- Bendict's solution:

This is a modification of Fehling's solution. To 3 ml of Bendict's solution, add 2 ml of dil. soln. of carbohydrate, boil for 2 minutes and allow to cool.

Observation	Inference
A red Cu_2O ppt.	Reducing sugar
Clear solution	Non reducing sugar



Bendict reagent

3-Tollen's reagent (Ammonical silver nitrate)

Add 1 ml of Tollen reagent to 1ml of carbohydrate soln. and heat gently.

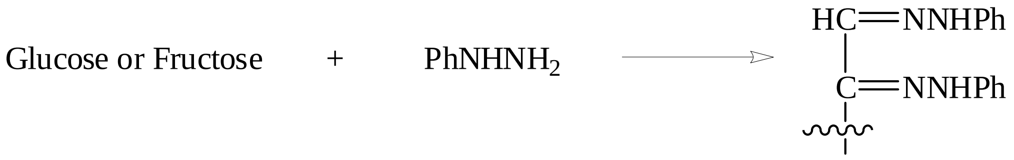
Observation	Inference
A silver mirror or black ppt. on cold or after gentle heating	Monosaccharide.
A silver mirror on heating.	Lactose or maltose.
-ve.	Sucrose.

$$\text{Glucose} + (Ag(NH_3)_2)OH \longrightarrow \text{Gluconic acid} + Ag\downarrow$$

4.Osazone formation:

Carbohydrates react with phenylhydrazine to give crystalline compounds known as osazone. Such compounds are used to identify the different types of carbohydrates by observing the crystalline form of the osazone under the microscope. This experiment is used to differiate between maltose and lactose.

Dissolve 0.2 g of the carbohydrate, 0.4g of phenylhydrazine hydrochloride and 0.6g of sod. acetate in 4 ml of water and dip the tube in a beaker containing boiling water and leave it as such for 20 minutes then place it a side to cool slowly. The osazone separates as a yellow crystalline precipitate.



$$\text{Glucose or Fructose} + PhNHNH_2 \longrightarrow \begin{array}{l} HC{=}NNHPh \\ \;| \\ \;C{=}NNHPh \\ \;| \end{array}$$

Osazone derivatives
Glucosazone or Fructosazone

Maltose **Lactose**

N.B. *this experiment is used to diffentiate between maltose and lactose.*

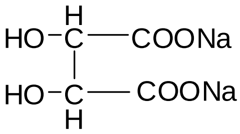
V.3. Salts of acids

All salt of acids (aliphatic and aromatic) are soluble in water, they are two types of acid salts (sodium and ammonium salts).

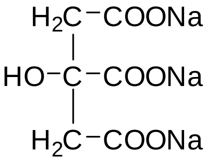
V.3.1. Sodium salts:

Aliphatic salts of acids:

Sod. Oxalate

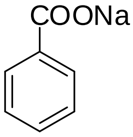


Sod. Tartrate

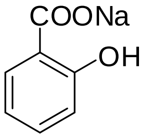


Sod. Citrate

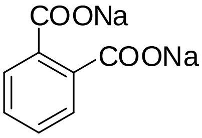
Aromatic salts of acids:



Sod. Benzoate



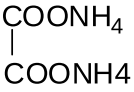
Sod. Salicylate



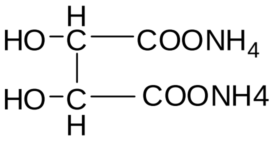
Sod. Phthalate

V.3.2. Ammonium salts:

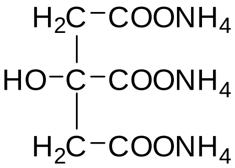
Aliphatic salts of acids:



Amm. Oxalate

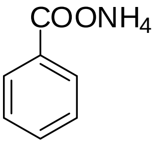


Amm. Tartrate

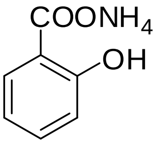


Amm. Citrate

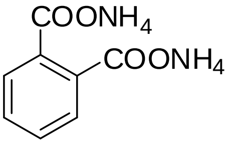
Aromatic salts of acids:



Amm. Benzoate



Amm. Salicylate



Amm. Phthalate

V.3.3. Differentiation between sod. and amm. Salts:

V.3.3.1. Odour test:

Add 1ml of NaOH soln. to a portion of unknown solid substance in a test tube and shake well, if odour of ammonia is observed , then the salt is Ammonium salt of acid. If no odour of ammonia (-ve), then we have Sodium salt of acid.

The following combinations of mixtures be considered :

I- Acid + Acid
II- Acid + Base
III- Acid + Phenol
IV- Base + phenol
V- Acid + Neutral
VI- Base + Neutral
VI- Phenol + Neutral
VIII- Neutral + Neutral

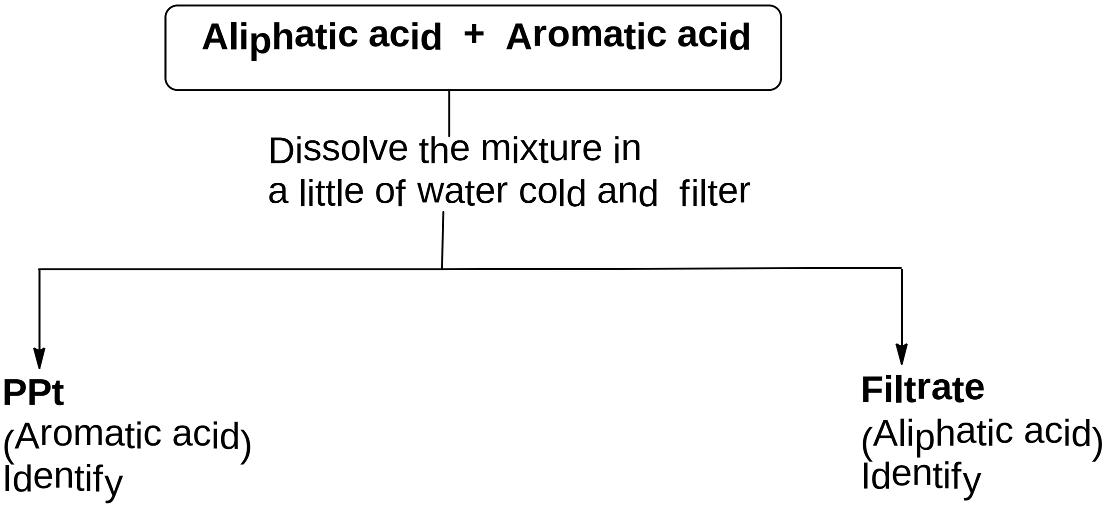
Mixture (I): Acid + Acid

This class of mixture includes **three** possibilities:

i- Aliphatic acid + Aliphatic acid (Completely soluble in water)
ii- Aromatic acid + Aromatic acid (Completely insoluble in water)
iii- Aliphatic acid + Aromatic acid (partially soluble in water)

The first and second mixtures could not be separated using our practical techniques and those mixtures required chromatographic techniques for separation.

In this study we will focus on the mixture (iii):

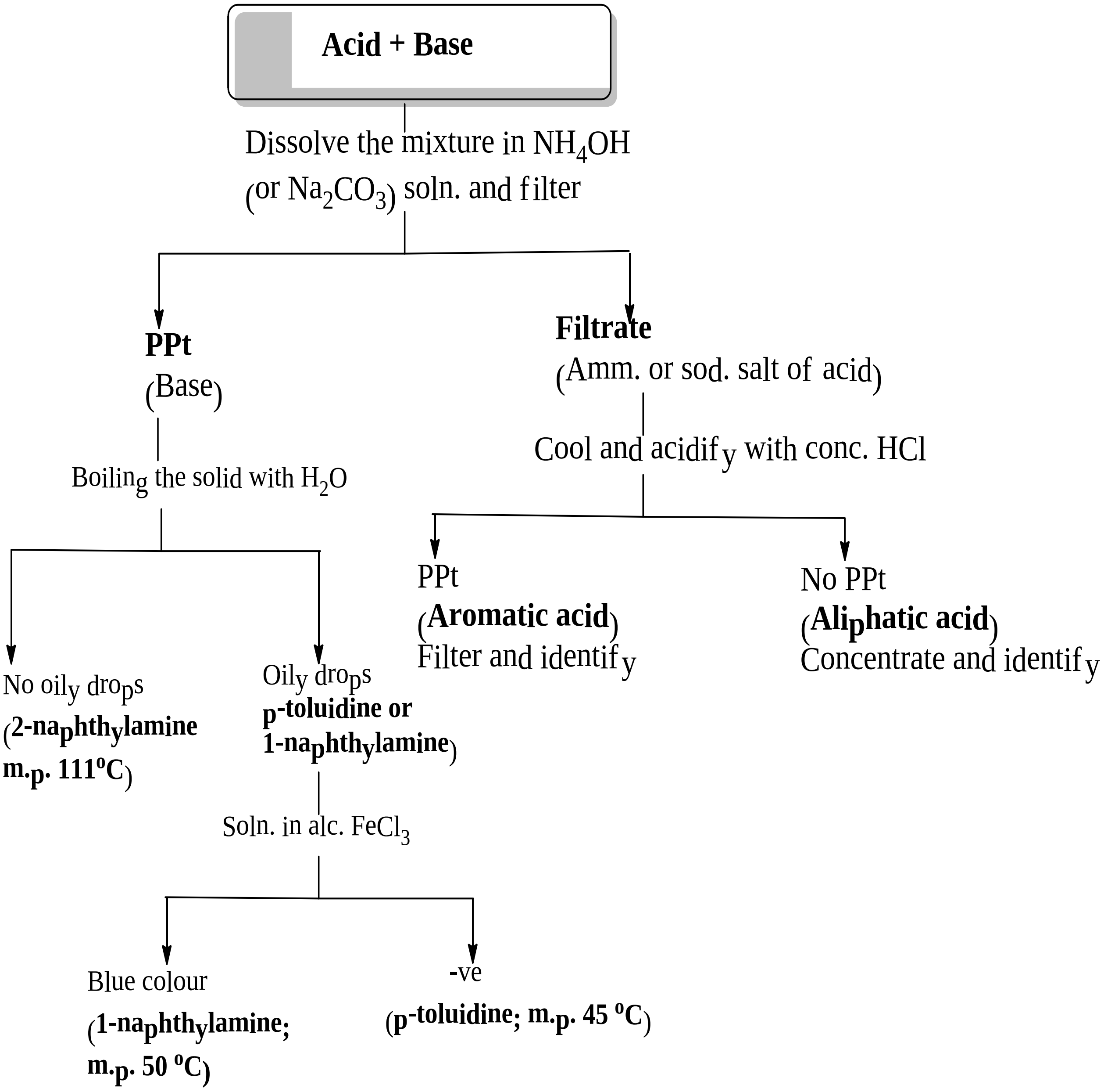


Mixture (II): Acid + Base

The acid may be aliphatic or aromatic. Thus two types are possible:

I- Base + Aliphatic acid
II- Base + aromatic acid

To identify each type carry out the following:



Mixture (III): Acid + Phenol

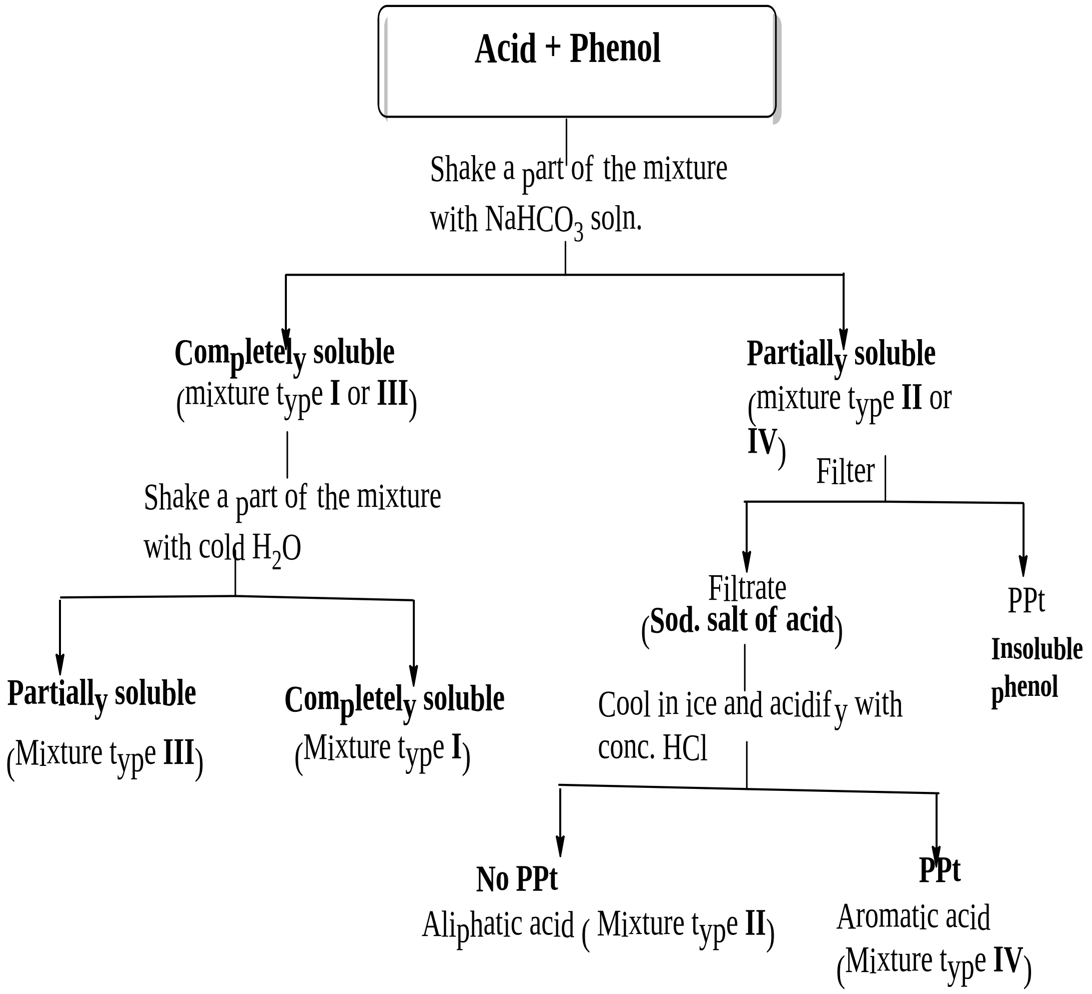
The acid may be aliphatic or aromatic. The phenol may be water soluble or water insoluble so, four types are possible:

(I) Aliphatic acid + water soluble phenol

(II) Aliphatic acid + water insoluble phenol

(III) Aromatic acid + water soluble phenol

(IV) Aromatic acid + water insoluble phenol



Type (I) Aliphatic acid + water soluble phenol

Dissolve in $NaHCO_3$ soln. Extract with ether in separating funnel

Ethereal layer
(water soluble phenol)
Evaporate on water
Bath till dryness, identify the
Residue (phenol)

Aqueous layer
(sod. Salt of acid)
acidify with dil.HCl,
concentrate, (identify)

Type(II) Aliphatic acid + water insoluble phenol

Dissolve the mixture in cold water, and filter

PPt

Water insoluble phenol

Alc. Soln + $FeCl_3$

Greenish violet

(α-Naphthol)

Faint green

(β-Naphthol)

Filterate

Aliphatic acid

Identify

Type (III) Aromatic acid + water soluble phenol

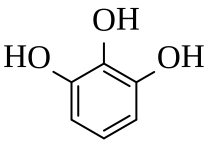
Dissolve the mixture in water, and filter

PPT
Aromatic acid
(Identify)

Filterate
water Soluble phenol

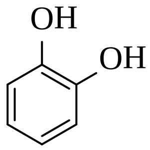
Soln. (a part of the filtrate) + $FeCl_3$

Red → NaOH → Violet



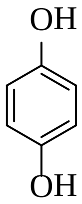
Pyrogallol; m.p. 132 °C

Green → NaOH → Red



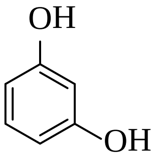
Catechol; m.p. 105 °C

Red → NaOH → Dark green crystals



Ouinol; m.p. 170 °C

Violet colour



Resorcinol; m.p. 110 °C

Type (IV) Aromatic acid + water insoluble phenol

Dissolve in $NaHCO_3$ soln. and filter

PPt

Water- insoluble phenol

(Identify)

Filterate

Sod. Salt of acid

Cool and acidify with dil. HCl,

Identify the ppt, (Aromatic acid)

Mixture (IV):Base + Phenol

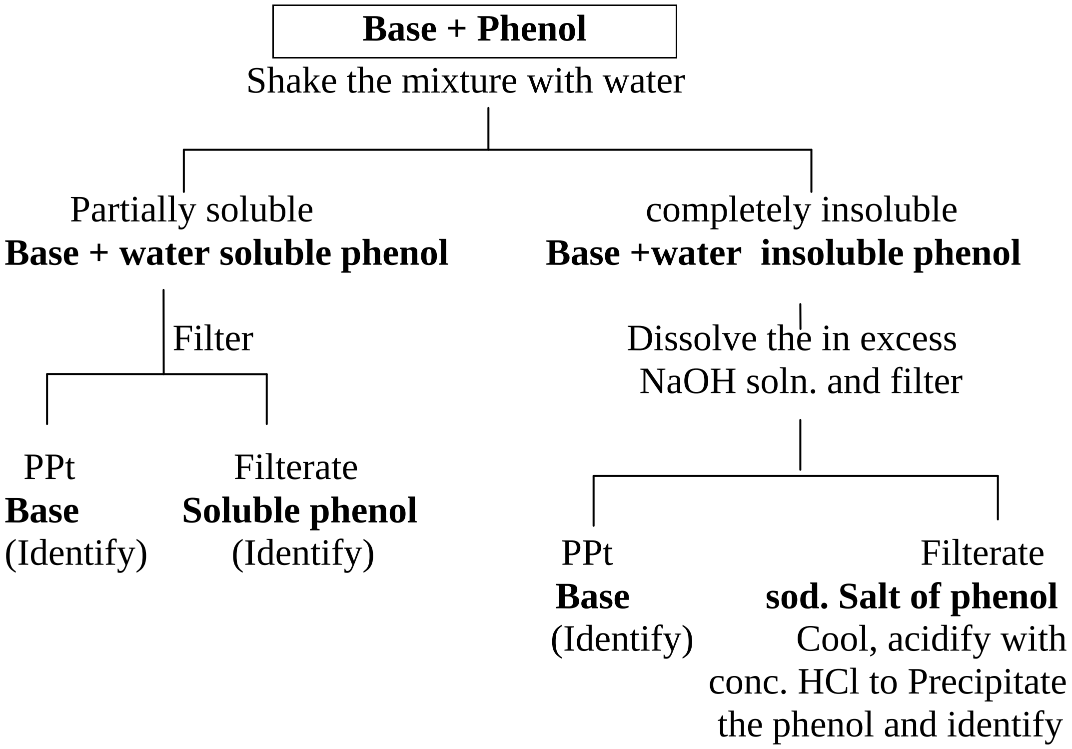
The phenol may be water soluble phenol or water insoluble.

Two types are possible:

(I) Base + water soluble phenol.

(II) Base + water insoluble phenol.

To know the type and the components of each type:



Mixture (V): Acid + Neutral

The acid may be aliphatic or aromatic. The neutral may be carbohydtrate (starch, and any sugar), salt of acid, or insoluble neutral as hydrocarbons (naphthalene and anthracene).

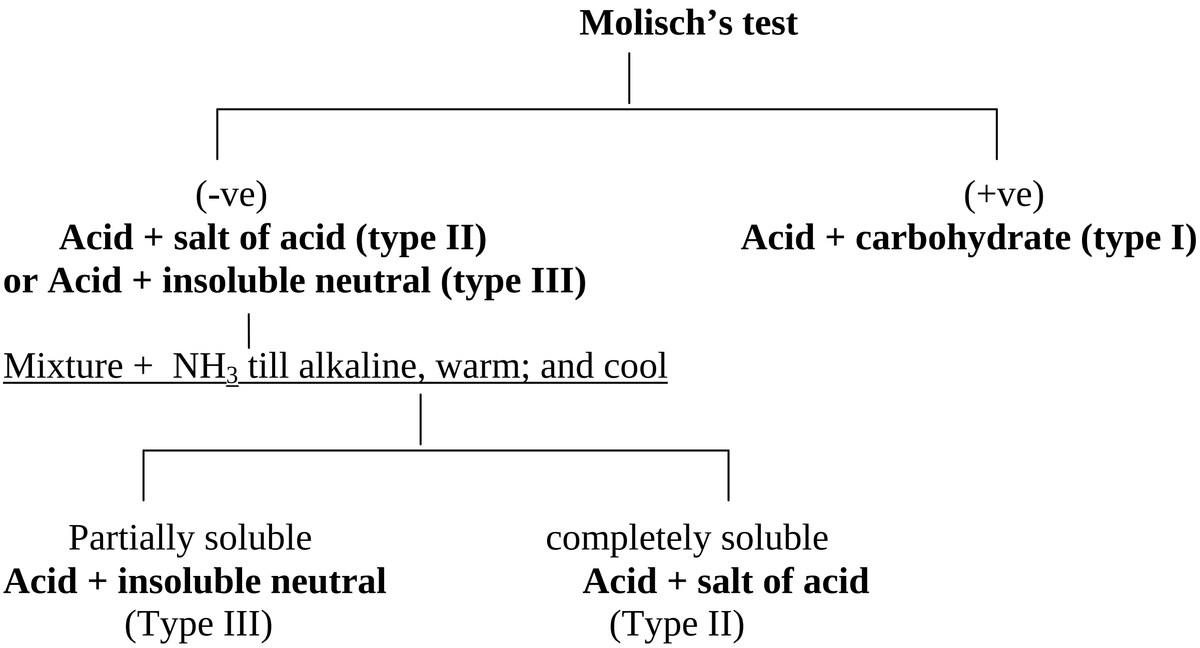
Thus three types are possible:

(I) Acid + carbohydrate

(II) Acid + salt of acid

(III) Acid + insoluble neutral

To identify the type of the mixture, carry out Molisch's test on a part of the mixture:



Molisch's test

(-ve)
Acid + salt of acid (type II)
or Acid + insoluble neutral (type III)

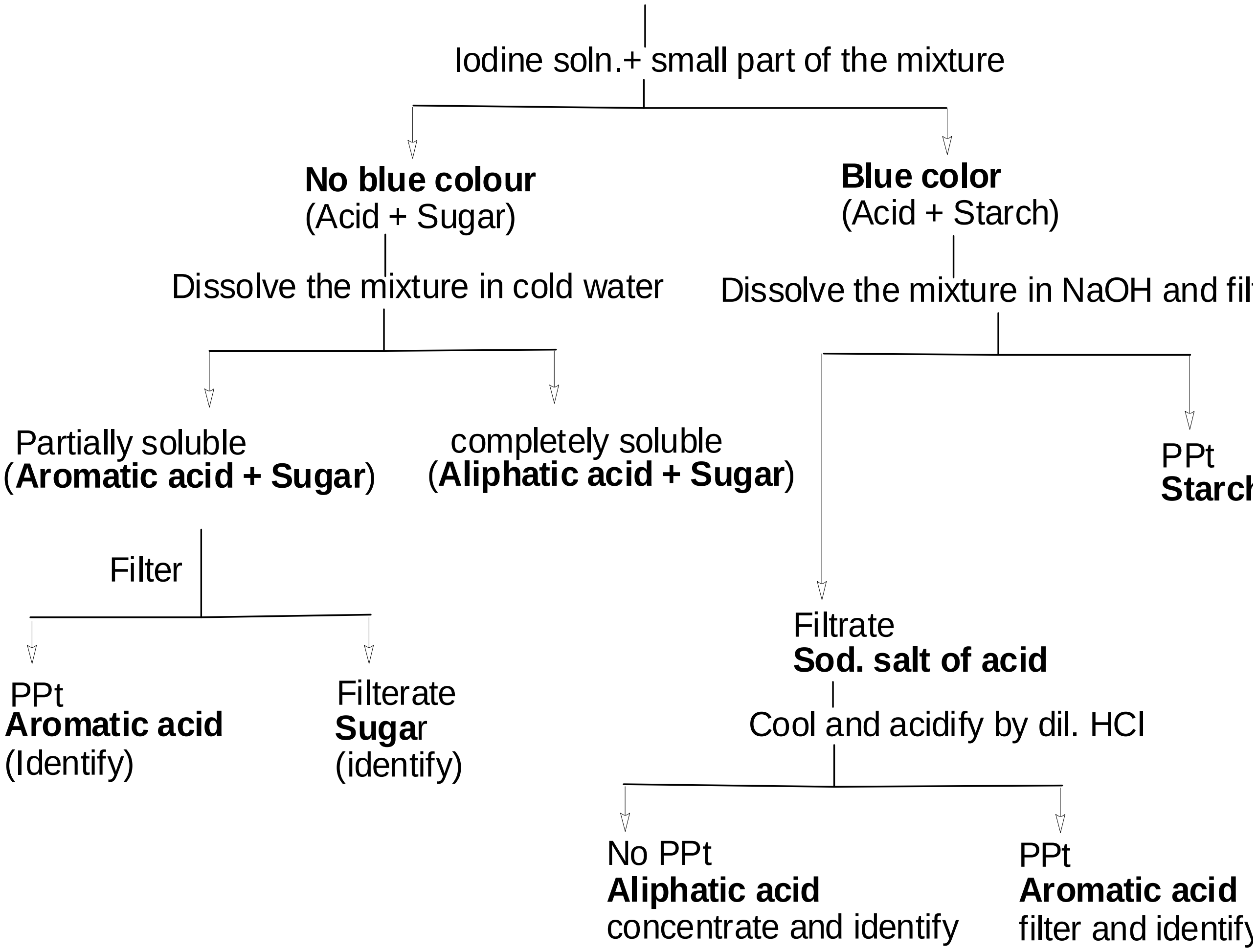
(+ve)
Acid + carbohydrate (type I)

Mixture + NH_3 till alkaline, warm; and cool

Partially soluble
Acid + insoluble neutral
(Type III)

completely soluble
Acid + salt of acid
(Type II)

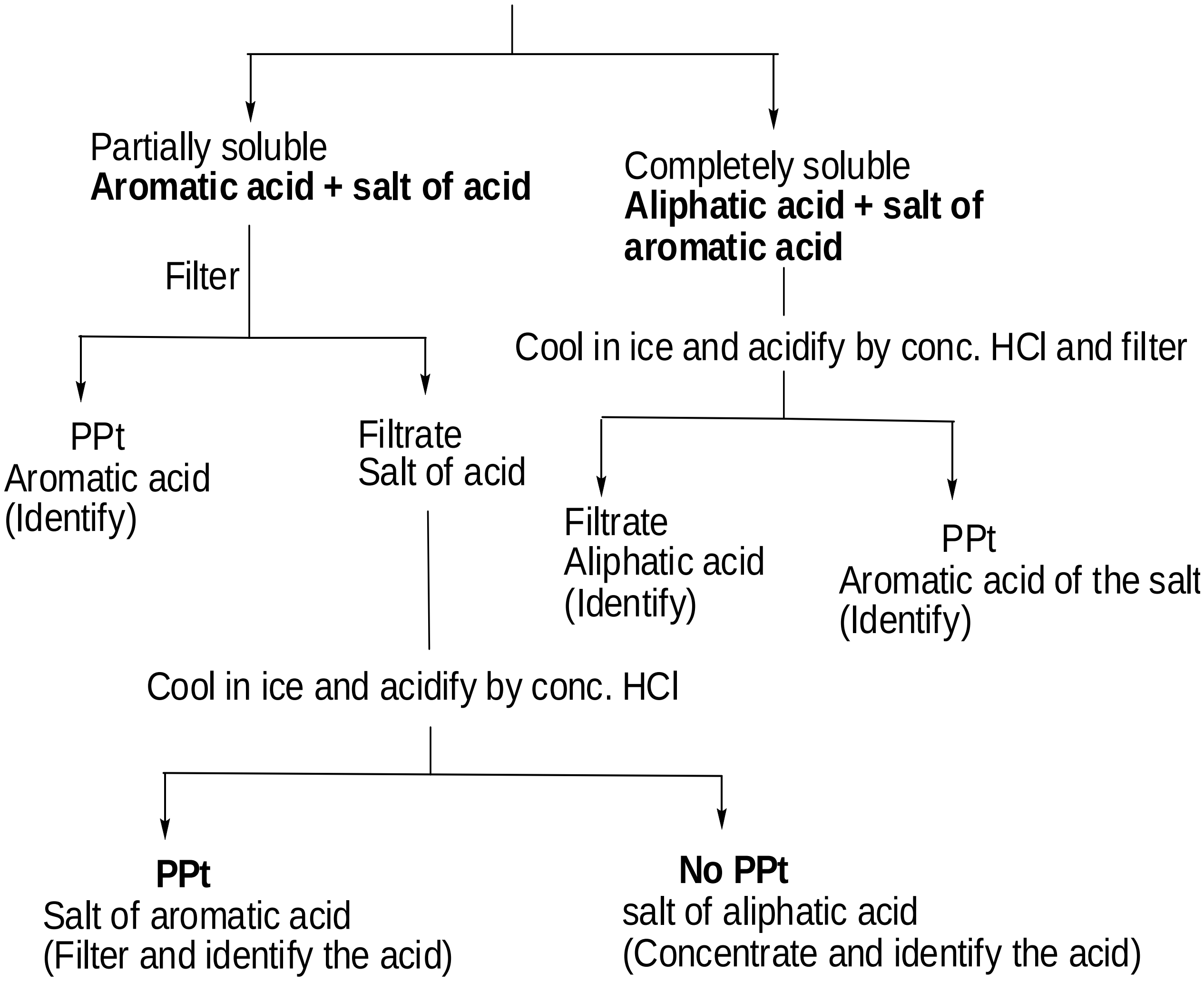
Type (I) Acid + Carbohydrate



Type (II): Acid + Salt of acid

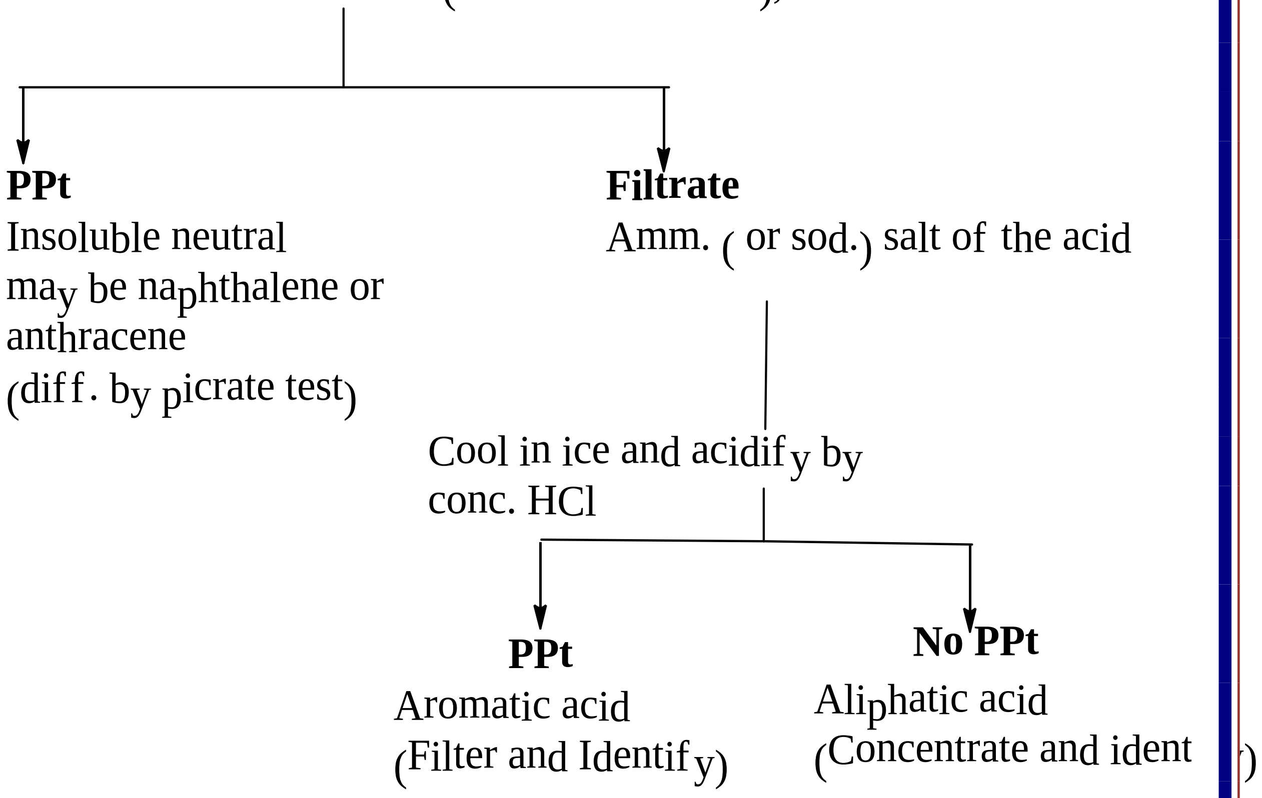
(1) Boil a part of the mixture with NaOH soln. if NH_3 evolved, then we have ammonium salt.

(2) Shake all the mixture with cold water.



Type (III): Acid + insoluble neutral

the mixture in excess ammonia (or NaHCO3 soln.), then filter



PPt

Insoluble neutral
may be naphthalene or anthracene
(diff. by picrate test)

Filtrate

Amm. (or sod.) salt of the acid

Cool in ice and acidify by conc. HCl

PPt

Aromatic acid
(Filter and Identify)

No PPt

Aliphatic acid
(Concentrate and ident y)

Mixture(VI): Base + Neutral

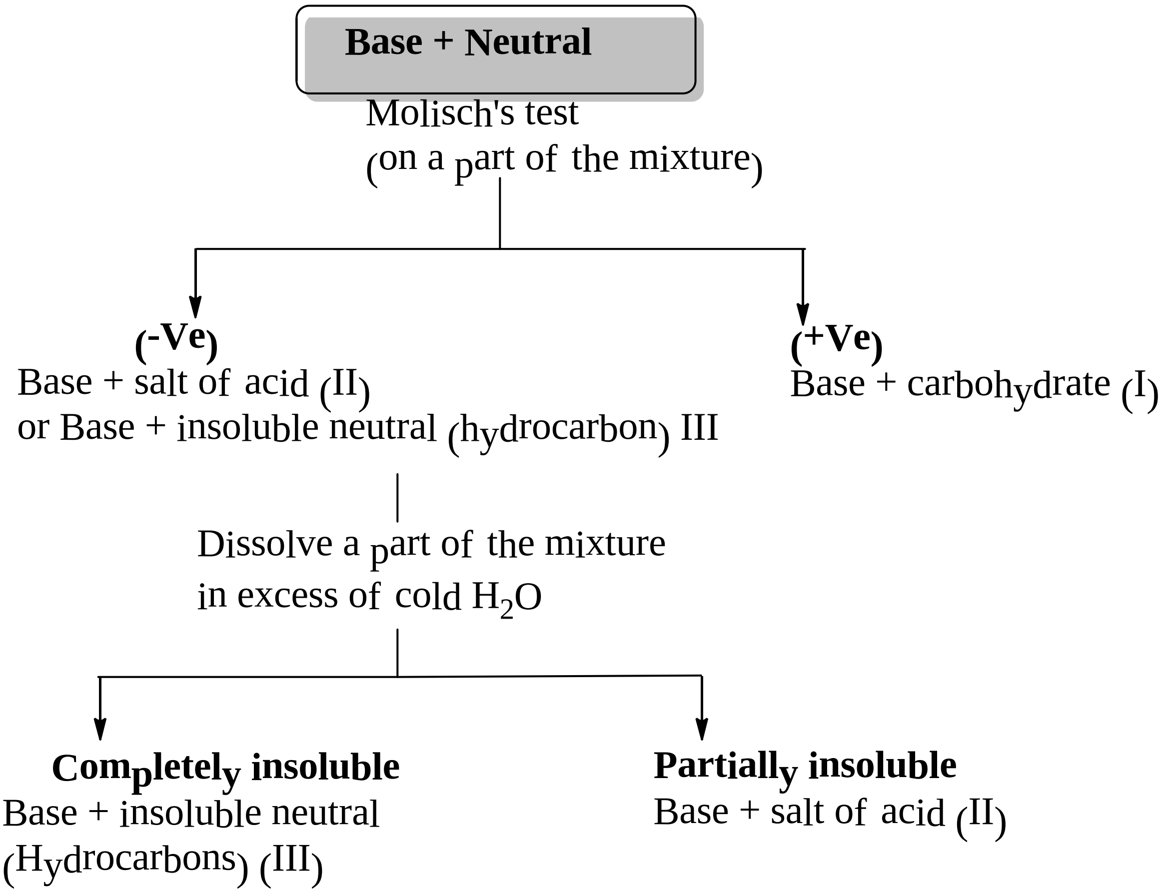
Three types are possible:

(I) Base + carbohydrate (sugar or starch)

(II) Base + salt of acid

(III) Base + hydrocarbon (insoluble neutral)

To know the type of the mixture, carry out the following:



Type (I): Base + carbohydrate

Iodine test

-ve
No blue colour
Base + sugar
Dissolve in water
and filter

Filtrate
Sugar
(identify)

PPt
Base
(Identify)

+ve
blue colour
Base + starch
Add HCl
and filter

Filtrate
Base HCl
Add NaOH
Base
(identify)

PPt **Starch**

Type (II): Base + salt of acid

(1) Boil a part of the mixture with NaOH soln. if NH_3 evolves then we have ammonium salt.

(2) Dissolve all the mixture in hot water, cool in ice and then filter

Filtrate
salt of acid
Cool in ice and acidify by conc. HCl

No ppt
Salt of aliphatic acid
(Concentrate and identify the acid)

ppt
Salt of aromatic acid
(Filter and identify the acid)

PPt
Base
(Identify)

Type (III): Base + Hydrocarbon

Dissolve all the mixture in hot dil. HCl and filter while hot

Filtrate

Base as hydrochloride

(hydrocarbons)

Cool in ice and add NaOH
(Identify the free base)

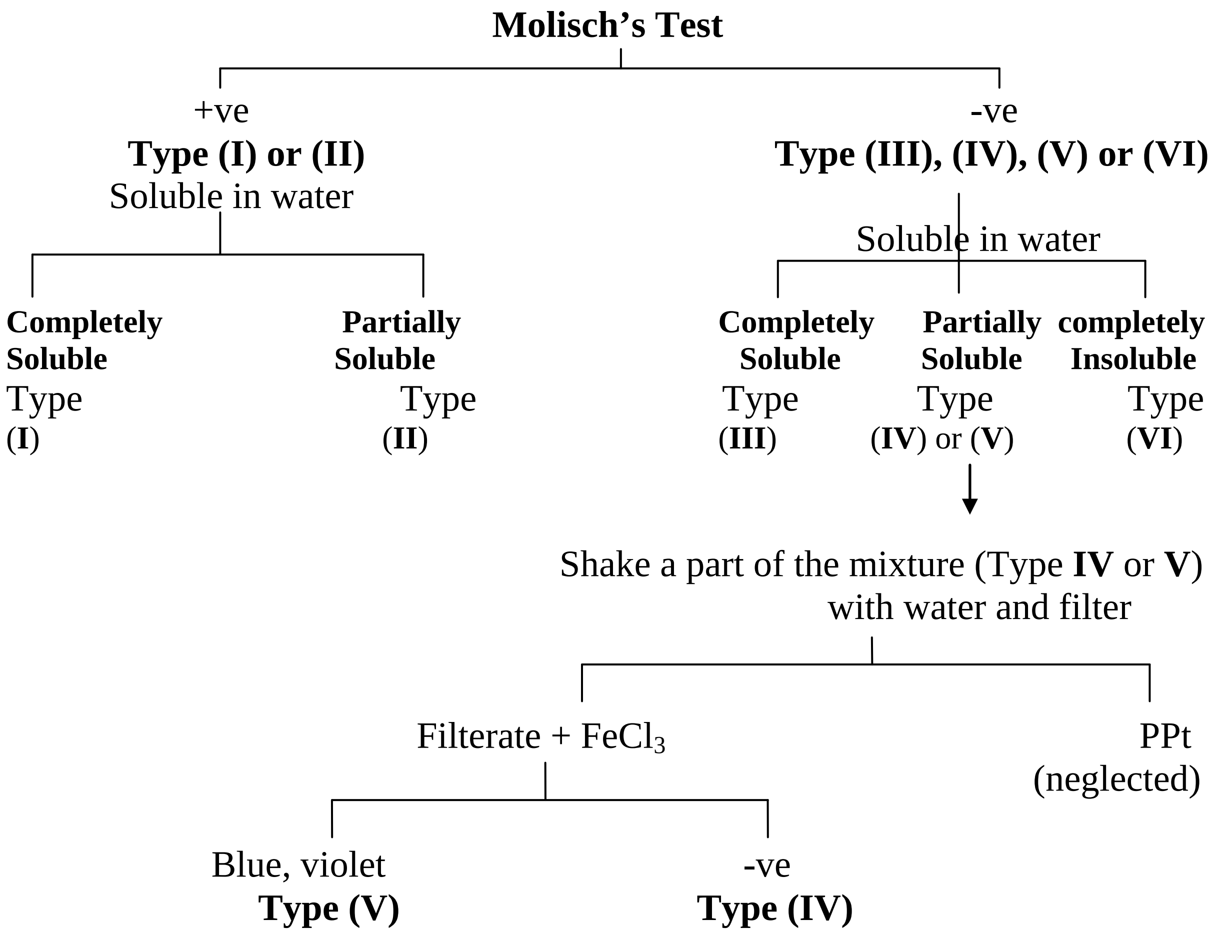
PPt

Insoluble neutral

Idrntify as picrates
(naphthalene or anthracene)

Mixture (VII): Neutral + Phenol

(I) Sol. phenol + carbohydrate
(II) Insol. phenol + carbohydrate
(III) Sol. phenol + salt of acid
(IV) Insol. phenol + salt of acid
(V) Sol. phenol + Insol.Neutral(hydrocarbon)
(VI) Insol. phenol + Insol. Neutral (hydracarbon)



Type (I) water-soluble phenol + carbohydrate

Iodine test

-ve

Sol. Phenol + sugar

Identify each in the presence of other

+ve

Sol. Phenol + starch

Dissolve in water
and filter

Sol. Phenol
(identify)

PPt (starch)

Type (II): Water-insoluble phenol + carbohydrates

Iodine test

No blue colour

Insoluble phenol + sugar
Dissolve in hot water
and filter

Filtrate
Sugar
(identify)

PPt
Insoluble Phenol
(identify)

blue colour

Insoluble phenol + starch

Type (III): Water-soluble phenol + Salt of aromatic acid

Dissolve the mixture in water, add conc. HCl; and filter

Filterate
Soluble phenol
(identify)

PPt
Aromatic acid
(identify)

Type (IV): Water-insoluble phenol + salt of acid

Dissolve the mixture in water; and filter

- Filterate
  Salt of acid
  Part of the filterate + Conc. HCl
  - No ppt
    Aliphatic acid
  - ppt
    Aromatic acid
- PPt
  Insoluble phenol
  (identify)

Type (V): Soluble phenol + Insoluble neutral

Dissolve the mixture in water; and filter

- Filterate
  Soluble phenol
  (identify)
- PPt
  Insoluble neutral
  (identify)

Type (VI): Insoluble phenol + Insoluble neutral

Dissolve the mixture in NaOH; and filter

- Filterate
  Sod. Salt of phenol
  ↓ HCl
  PPt
  Insoluble phenol
  (Identify)
- PPt
  Insoluble neutral

Mixture (VIII): Neutral + Neutral

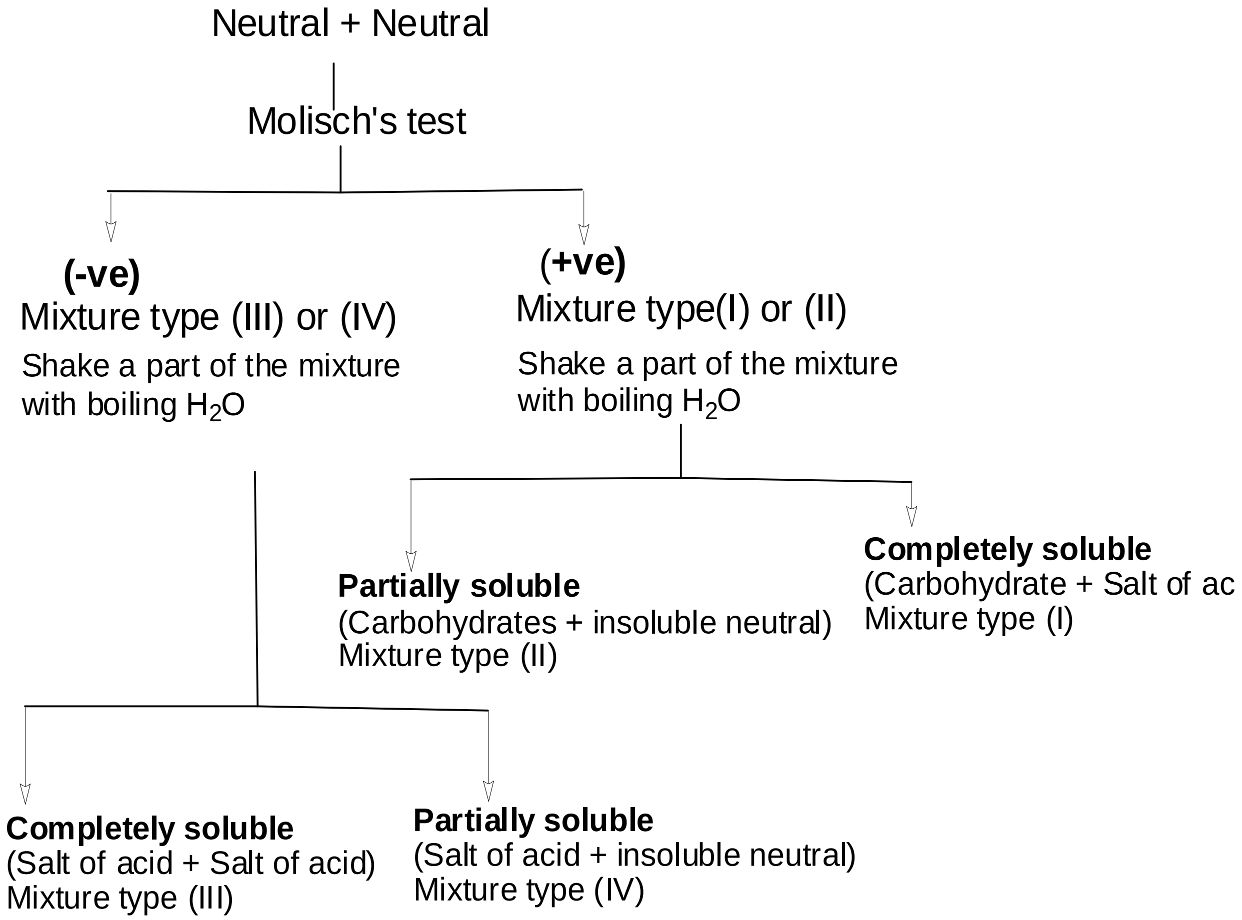
The neutral substance may be carbohydrate, salt of acid, insoluble neutral (hydrocarbons). Four types are possible:

(I) Carbohydrate + salt of acid

(II) Carbohydrate + Insoluble neutral (hydrocarbon)

(III) Salt of aliphatic acid + salt of aromatic acid

(IV) Salt of acid + Insoluble neutral (hydrocarbon)



(I) Carbohydrates + Salt of acid

Boil a part of the mixture with NaOH if ammonia odour is evolved then we have ammonium salt.

Part of the mixture + NaOH (heat)

-ve
Metallic salts

NH_3 odour
Amm. salt

Shake a part of the mixture with iodine soln.

Iodine test

No blue colour
Sugar + Salt of acid
Identify the sugar in the mix.

Dissolve all the mixture

in water and add Conc. HCl

No ppt
starch

Aliphatic acid
(Salt of aliphatic acid)

(Identify)

ppt

Aromatic acid
(Salt of aromatic acid)

(Filter , then identify)

blue colour

Starch + salt of acid

Dissolve all the mixture
in cold H_2O and filter

Filtrate

Salt of acid

PPt

Cool in ice and Acidify with Conc.HCl

No PPt
Free aliphatic acid
(Salt of aliphatic acid)
concentrate (Identify)

ppt
Free aromatic acid
(Salt of aromatic acid)
filter (Identify)

Type (II): Carbohydrate +Insoluble neutral (hydrocarbon)

Iodine test

No blue colour

Sugar + Hydrocarbon

Dissolve in boiling water, cool in ice and filter

Filterate

Sugar

(Identify)

PPt

Hydrocarbon

(Identify as picrate)

Blue colour

Starch + Hydrocarbon

Boil in dil.HCl tohydrolyse starch , and cool in ice

Filterate

Sugar

PPt

Hydrocarbon

Type (III): Salt of Aliphatic Acid + Salt of Aromatic acid

One salt is of aliphatic acid, the other should be salt of aromatic.

1) Test for amm.salt by boiling with NaOH, if odour of NH_3 , the the salt is ammonium salt.

2) Dissolve in H_2O, cool in ice then acidify by conc. HCl and filter

Filterate

Salt of aliphatic acid

(Concentrate and identify the acid)

PPt

Salt of aromatic acid

(Filter the acid and identify)

Type (IV): Insoluble neutral (hydrocarbon) + Salt of acid

1) Boil a part of the mixture with NaOH, if NH_3 evolved, then we have ammonium salt.

-ve
Metallic salt

NH_3 odour
Amm. salt

2) Dissolve all the mixture in hot water, boil and then filter.

Dissolve in cold water and filter

Filterate
Salt of acid

PPt
Hydrocarbon

Part of the filtrate
+ Conc. HCl

(Identify)

No PPt
Salt of aliphatic acid
(Concentrate and
Identify the acid)

PPt
Salt of aromatic acid
(Filter and identify
the aromatic acid)

Extraction

Extraction is a technique commonly used in organic chemistry to separate a material you want from those you do not. While the term extraction may be unfamiliar to you, the process is actually something you commonly perform. For example, many of you probably start the day, especially after a long night of studying, with an extraction when you brew a pot of coffee or tea. By heating coffee grounds or tea leaves with hot water, you extract the caffeine, together with other water-soluble compounds such as dark-colored tannins, from the solid material. You can then drink the liquid, which is certainly more enjoyable than eating coffee grounds or tea leaves, to ingest the caffeine and benefit from its stimulating effect. Similarly, when you make a soup, the largely aqueous liquid portion contains numerous organic and inorganic compounds that have been extracted from spices, vegetables, fish, or meat, and these give your culinary creation its distinctive flavor. In the procedures found in this chapter, you will have an opportunity to develop your existing experimental skills further by isolating organic compounds sing different types of extractions.

INTRODUCTION

The desired compound from a reaction is frequently part of a mixture, and its isolation in pure form can be a significant experimental challenge. Two of the more common methods for separating and purifying organic liquids and solids are **recrystallization** and **distillation;** Two other important techniques available for these purposes are **extraction** and **chromatography.** Both of these methods involve partitioning of compounds between two *immiscible* phases. This process is termed **phase distribution** and can result in separation of compounds if they distribute differently between the two phases. Distribution of solutes between phases is the result of **partitioning** or **adsorption** phenomena. Partitioning involves the difference in solubilities of a substance in two immiscible solvents in other words, *selective dissolution*. Adsorption, on the other hand, is based on the *selective attraction* of a substance in a liquid or gaseous mixture to the surface of a solid phase. The various chromatographic techniques depend on both of these processes, whereas the process of extraction relies only on partitioning. Extraction involves *selectively* removing one or more components of a solid, liquid, or gaseous mixture into a separate phase. The substance being extracted will partition between the two immiscible phases that are in contact, and the ratio of its distribution between the phases will depend on the relative solubility of the solute in each phase.

I. Liquid-liquid extraction is one of the most common methods for removing an organic compound from a mixture. This process is used by chemists not only in the isolation of natural products but also in the isolation and purification of products from most chemical reactions. The technique involves distributing a solute, A, between two immiscible liquids, S_x, the **extracting phase,** and S_o, the **original phase.** The immiscible liquids normally encountered in the organic laboratory are water and some organic solvent, such as diethyl ether, $(C_2H_5)_2O$, or dichloromethane, CH_2Cl_2. At a given temperature, the amount of A, in g/mL, in each phase is expressed *quantitatively* in terms of a constant, K, commonly called the **partition coefficient** (Eq.1). Strictly speaking, the volumes of *solution* should be used in the definition of [A], but if the solutions are dilute, only slight errors result if volumes of *solvent* are used. Furthermore, a close *approximation* of the partition coefficient K may be obtained by simply dividing the solubility of A in the extracting solvent S_x by the solubility of A in the original solvent S_o.

$$K = \frac{[A] \text{ in } S_x}{[A] \text{ in } S_o} \qquad \text{(Eq.1)}$$

Selection of the appropriate extracting solvent is obviously a key for successfully using this technique to isolate and purify compounds, and important guidelines for making the correct choice are summarized here.

1. The extracting solvent *must not react* in a chemically irreversible way with the components of the mixture.

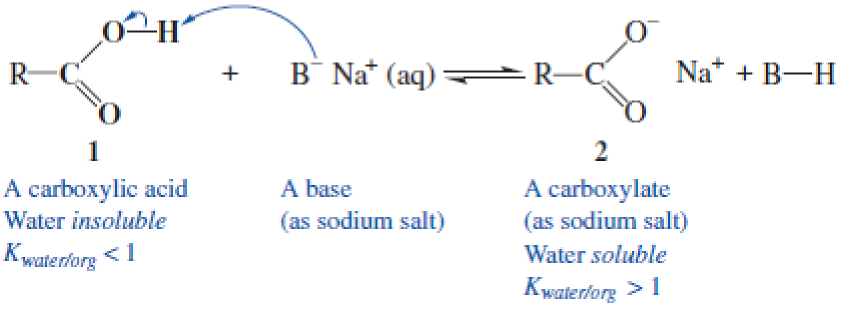
2. The extracting solvent *must be immiscible,* or nearly so, with the original solution.

3. The extracting solvent *must selectively remove* the desired component from the solution being extracted. That is, the partition coefficient *K* of the component being removed must be *high,* while the partition coefficients of all other components should be *low.*

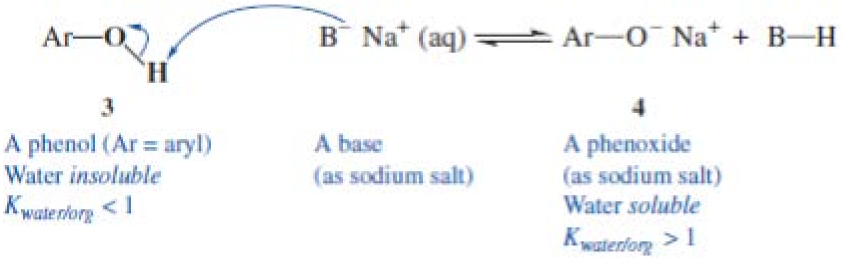
4. The extracting solvent *should be readily* separable from the solute. Use of a volatile solvent facilitates its removal by simple distillation.

Base and acid extractions

Now consider what happens if a mixture of *two or more* compounds is present in a given volume of solvent S_o and an extraction using a solvent S_x is performed. If the partition coefficient *K* of one component, *A*, is *significantly* greater than 1.0 and if those of other components are *significantly* less than 1.0, the majority of *A* will be in S_x, whereas most of the other compounds will remain in S_0. Physical separation of the two solvents will give a partial separation, and thus purification, of the solute *A* from the other components of the mixture. Solutes differing significantly in polarity should have very different coefficients *K* for partitioning between nonpolar and polar solvents. For example, consider the distribution of two organic compounds, the first neutral and nonpolar, and the second ionic and polar, between a nonpolar solvent and a polar solvent. If a solution of these compounds in the nonpolar solvent is shaken with the polar solvent, the neutral compound will preferentially partition into the *nonpolar* phase, whereas the polar constituent will preferentially partition into the *polar* phase. Separating the two phases effects a separation of the two solutes. Carboxylic acids and phenols (**1** and **3**) are two classes of organic compounds containing **functional groups** that are **polar** and **hydrophilic** (water-loving). Unless they contain fewer than about six carbon atoms, such compounds are generally either *insoluble* or only *slightly soluble* in water because of the **hydrophobic** (water-avoiding) properties of the carbon containing portion, R or Ar, of the molecule. They are *soluble* in common organic solvents like dichloromethane or diethyl ether that have at least modest polarity. Consequently, carboxylic acids or phenols dissolved in diethyl ether, for example, will largely remain in that phase when the solution is extracted with water.

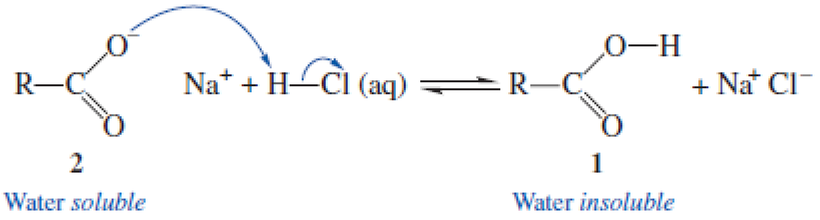


(Eq.2)

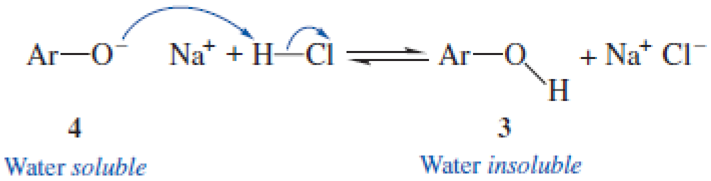


(Eq.3)

Now consider what happens if the organic solution is extracted with a *basic* aqueous solution. If the base, B–, is strong enough, the organic acid **1** or **3** will be converted into the corresponding **conjugate base 2** or **4** (Eqs. 2 and 3). Because it is a salt, the conjugate base is highly polar, and K water/org >1. Thus the conjugate bases of organic acids may be selectively extracted from an organic phase into an aqueous phase. If the basic extract is then neutralized by an acid such as hydrochloric acid, the conjugate base **2** or **4** will be protonated to regenerate the organic acid **1** or **3** (Eq. 4 and5). Because the acid is water-insoluble, it will appear as either a precipitate or a second layer, if it is a liquid. The desired organic acid may then be recovered by filtration or separation of the layers.



(Eq.4)



(Eq.5)

Thus, two water-insoluble organic compounds, **HA,** which is acidic, and **N,** which is neutral, that are dissolved in an organic solvent may be separated by selectively extracting the acidic compound into a basic aqueous phase. After the aqueous and organic phases are separated, **HA** is recovered from the aqueous phase upon neutralization, and **N** is obtained by removing the organic solvent (**Fig.1**). The choice of the base is determined by the acidity of the organic acid **HA.** This is defined in aqueous solution by $\boldsymbol{K_a}$ and is often expressed as $\mathbf{p}\boldsymbol{K_a}$ (Eq.6) .

$$K_{a(HA)} = \frac{[A^-][H_3O^+]}{[HA]}$$
$$pK_a = -\log_{10} K_a$$

(Eq.6)

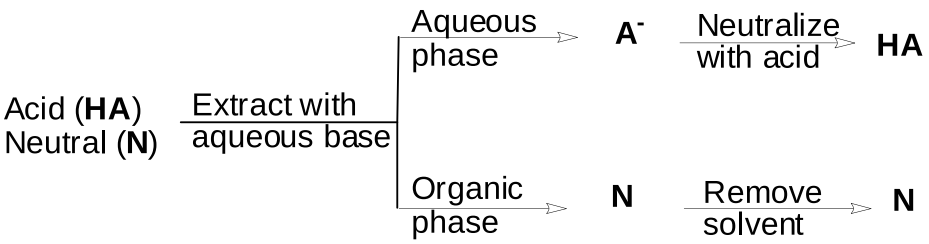


Fig.1; Separating an acidic compound and a neutral compound

The next step in determining what base is needed is to consider the equilibrium shown in equation 7, in which **HA** is the organic acid, **B–** is the base being used, and **HB** is the **conjugate acid** of this base. The **equilibrium constant,** K_{eq}, for this process is given by equation 8. Given the expressions for Ka for **HA** and **HB** (Eqs. 6 and 9), Equation 8 transforms to equation 10. From this equation we see that log10 of the equilibrium constant, K_{eq}, equals pK_a(**HB**) – pK_a(**HA**). We can predict how effective a particular base **B**– will be in converting an organic acid **HA** to its anion simply by knowing the relative acidities of **HA** and **HB.** The equilibrium for Equation 6 will lie to the right; namely, log10 K_{eq} will be > 1 whenever the acidity of **HA** is *greater* than that of **HB**.

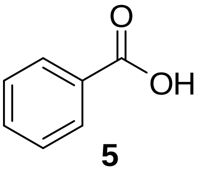
$$HA + B^- \xrightleftharpoons{K_{eq}} A^- + HB \qquad \text{(Eq.7)}$$

$$K_{eq} = \frac{[A^-][HB]}{[HA][B^-]} \qquad \text{(Eq. 8)}$$

$$K_{a(HB)} = \frac{[B^-][H_3O^+]}{[HB]} \qquad \text{(Eq. 9)}$$

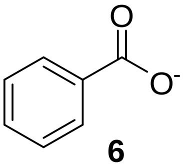
$$K_{eq} = \frac{K_{a(HA)}}{K_{a(HB)}} \text{ and } \log_{10} K_{eq} = \log_{10} K_{a(HA)} - \log_{10} K_{a(HB)} \qquad \text{(Eq.10)}$$

Let’s now consider two specific examples of mixtures of acidic and neutral compounds. Assume that you have a solution of benzoic acid (**5**) and naphthalene (**7**), a neutral compound, in diethyl ether and that you wish to separate the two. Both compounds and the solvent are insoluble in water at room temperature. The strategy is to convert **5** to its water-soluble conjugate base **6** by using an aqueous base to extract the organic solution. The pK_a of benzoic acid is 4.2, so any base whose *conjugate acid* has a pK_a *greater* than this will yield a value of log10 $K_{eq} > 1$ (Eq.10). Aqueous hydroxide, whose conjugate acid is water (pK_a 15.7), would give log10 K_{eq}=10.5, which means that this base will be extremely effective for the extraction of **5** into the aqueous phase. Indeed, aqueous hydroxide is commonly used for extracting organic acids into an aqueous phase.



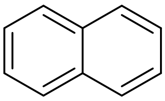
5

Benzoic acid



6

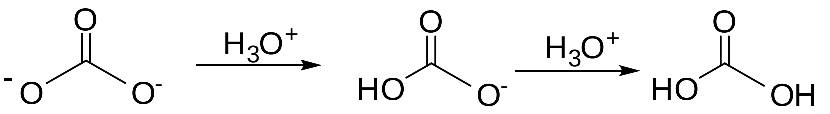
Benzoate



7

Naphthalene

Other aqueous bases could also be used. For example, carbonate (**8**) and bicarbonate (**9**) would also work because their conjugate acids, **9** and **10** (Eq.11), have p*K* as of 10.3 and 6.4, respectively. These bases are not as strong as hydroxide, so on the purely *thermodynamic* basis described equation 10, they would provide a lower value of log10 K_{eq}. However, both **8** and **9** may be converted to carbonic acid (**10**) upon di- and monoprotonation, respectively. Carbonic acid is *kinetically* unstable and decomposes to carbon dioxide and water, and this disrupts the equilibrium of equation 7, driving it to the *right* as **HB,** in the form of **10,** undergoes decomposition. The net result would be efficient deprotonation of **5** by these bases.



8 Carbonate

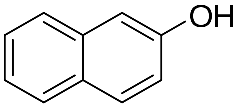
9 Bicarbonate

10 Carbonic acid

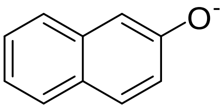
(Eq. 11)

$$H_2CO_3 \longrightarrow CO_2 + H_2O \qquad \text{(Eq. 12)}$$

It is important to recognize that the conversion of **10** into carbon dioxide and water presents a practical problem when **8** and **9** are used to deprotonate acids for purposes of extraction. If a separatory funnel is being used for the extraction, the gas pressure that results from the formation of carbon dioxide can blow out the stopcock and stopper, spraying the contents on you, your neighbors, or the floor. Alternatively, if a screw-cap vessel is being used, product may be ejected when the cap is loosened to relieve the gas pressure. Accordingly, it is preferable to use hydroxide for basic extractions as a general rule. Turning to a second example, assume you have a solution of 2-naphthol (**11**), which is a phenol, and naphthalene (**7**) in diethyl ether. The pKa of **11** is 9.5, so in this case either aqueous hydroxide or carbonate would give $K_{eq} > 1$; bicarbonate (**8**) is too weak a base to effect the deprotonation. Because aqueous hydroxide would provide $K_{eq} >> 1$, it is the base of choice for this extraction.

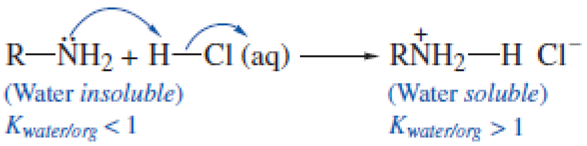


2-Naphthol

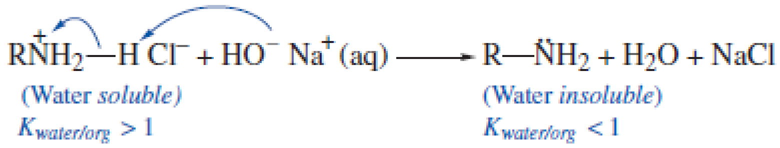


2-Naphthoxide

The examples discussed so far have involved separation of compounds having different acidities from neutral compounds. However, organic bases, usually amines, must sometimes be separated from mixtures containing organic acids and neutral compounds. Before considering how to perform such separations, it is useful to regard amines as being derivatives of ammonia where the hydrogen atoms on the nitrogen atom are replaced with substituted carbon atoms that are designated as R or Ar. Like the functional groups of carboxylic acids and phenols, the amino functional group is polar and hydrophilic, and amines having more than six carbon atoms are either insoluble or only slightly soluble in water because of the hydrophobic properties of the R or Ar groups. Amines are often soluble in aqueous acid at a pH < 4 because they are converted into their respective ammonium salts, the conjugate acids of the amines. This process is illustrated in equation 13. Wherein aqueous HCl serves as the acid. The enhanced ionic character of the ammonium salt, as we saw before with the salts of carboxylic acids and phenols, makes it water soluble ($K_{water/org}$ >1), whereas its parent base is not ($K_{water/org}$ <1). When the acidic solution is neutralized by adding aqueous base (Eq. 14), the ammonium ion is deprotonated and converted into the original water-insoluble organic base that will now either precipitate from solution or form a separate layer. The amine may then be recovered by filtration or by separation of the layers.



(Eq.13)



(Eq.14)

The question now is: How can the two water-insoluble organic compounds, **B,** which is basic, and **N,** which is neutral that are dissolved in an organic solvent be separated? This problem is analogous to the earlier one of separating two water-insoluble organic compounds, **HA,** which is acidic, and **N,** which is neutral (see Fig.1). Namely, the basic compound can be selectively extracted into an acidic aqueous phase as the soluble salt HB+. After the aqueous and organic phases are separated, **B** is recovered from the aqueous phase upon neutralization, and **N** is obtained by removing the organic solvent (Fig.2).

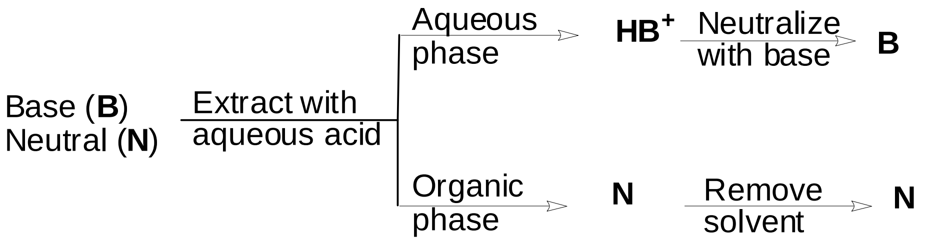
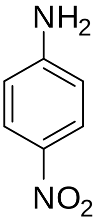
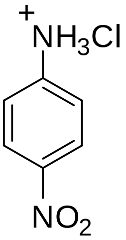


Fig.2; Separatig a basic compound and a neutral compound.

One example of an amine is 4-nitroaniline (**13**), which is converted into the corresponding anilinium ion **14** by aqueous hydrochloric acid. Although 4-nitroaniline is a weak base with a pK_b of 13.0, its conjugate acid **14** has a pK_a of 1.0 and is a relatively strong organic acid. Hence, it is necessary to use a strong aqueous acid such as 6 *M* HCl, in which the acid is actually the hydronium ion, H_3O+, having a pK_a of about –1.7, to convert **13** efficiently into its salt **14.**



13
4-Nitroaniline



14
4-Nitroanilinium hydrochloride

In the experimental procedures that follow, three types of separations of neutral, acidic, and basic compounds are described. The first involves separating a mixture of benzoic acid (**5**) and naphthalene (**7**), using aqueous hydroxide for the extraction. The flow chart for this separation corresponds to that of figure 1. The second procedure involves separating a mixture of **5, 7,** and 2-naphthol (**11**). In this case, **5** is first *selectively* removed from the solution by extraction with aqueous bicarbonate, which *does not* deprotonate **11.** A second basic extraction using aqueous hydroxide removes **11** from the organic solution. The flow chart for this sequence is depicted in figure 3. The last procedure involves separating a three-component mixture containing benzoic acid (**5**), naphthalene (**7**), and 4-nitroaniline (**13**). First **13** is *selectively* removed from the organic solution by extraction with aqueous hydrochloric acid, and then **5** is removed by extraction with aqueous base, leaving **7** in the organic solution. The flow chart for this sequence is depicted in figure 4. All procedures demonstrate the power of using the *pH*-dependence of water solubility of organic compounds as a basis for separation. Such procedures for the preparative separation of organic compounds are much easier than the chromatographic separations.

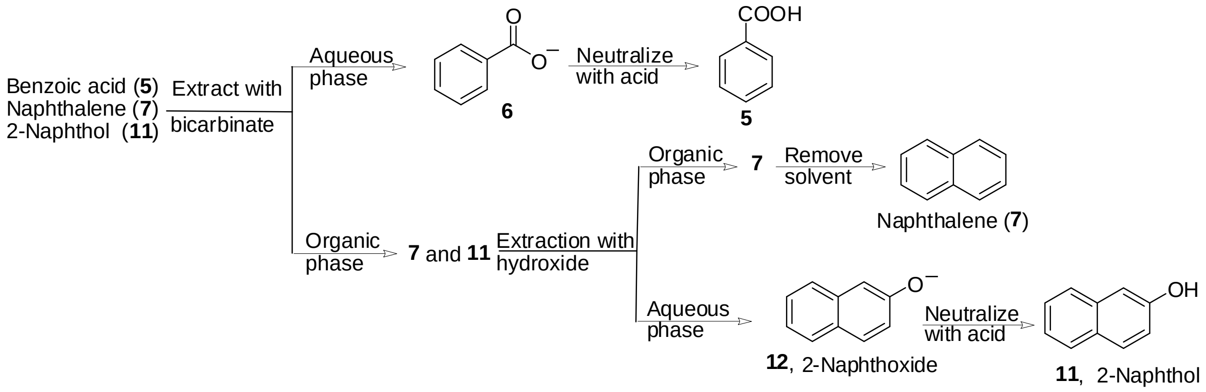


Fig.3. Separating a neutral compound and two compounds having different acidities.

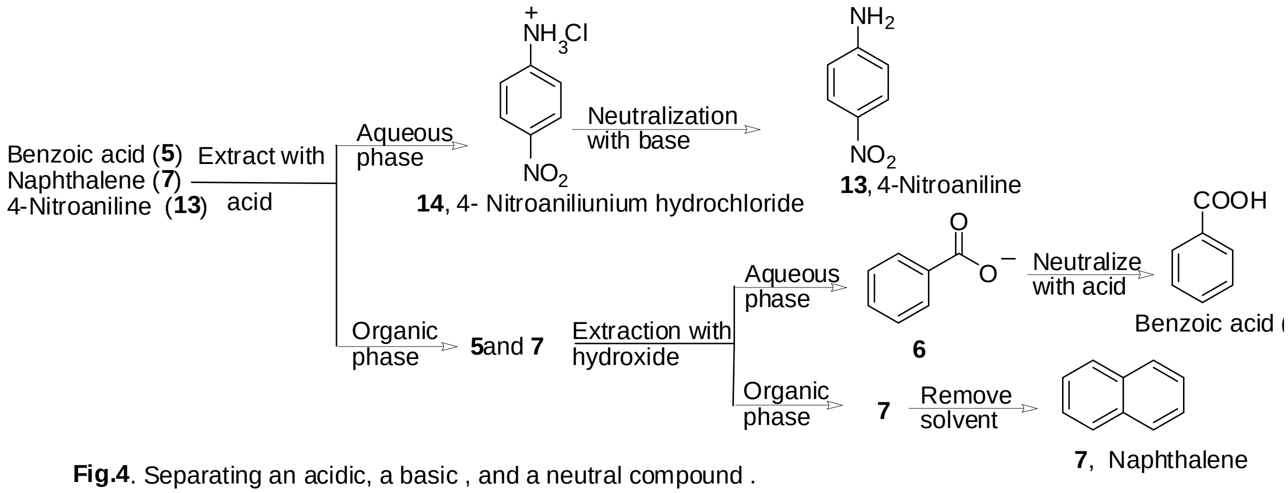
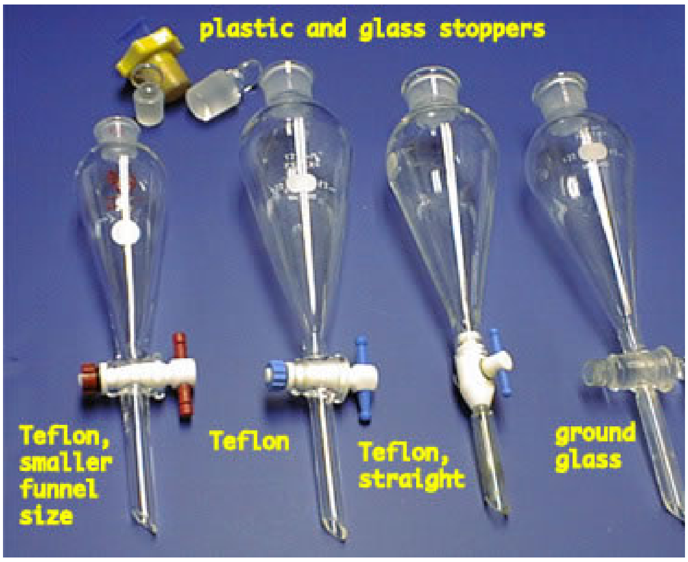
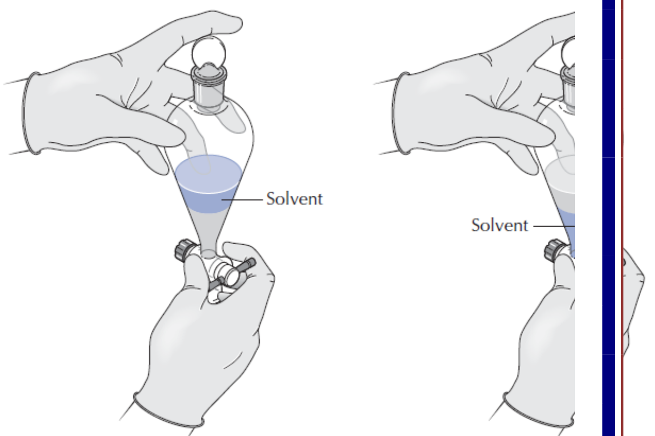


Fig.4. Separating an acidic, a basic , and a neutral compound .

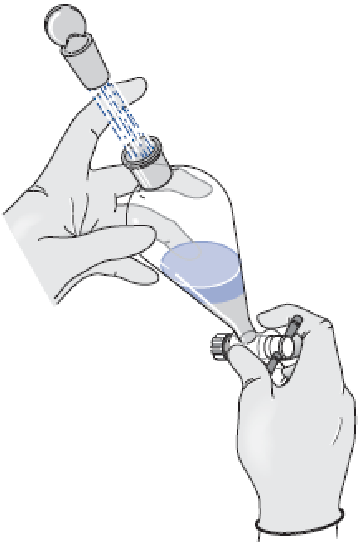
Using the separating funnel

1. Close the tap.
2. With the separating funnel supported in a ring clamp, add the two liquid phases and insert the stopper.
3. Remove funnel from ring clamp and, holding the stopper firmly with the palm of one hand, invert the funnel and release pressure through the tap.
4. After closing the tap, shake the funnel several times whilst holding both the stopper and the tap.
5. At frequent intervals during an extraction, release excess pressure through the tap. Take care not to point the stem, at your neighbor during this operation.
6. When the extraction is completed, replace the separating funnel in the ring clamp, remove the stopper and allow the phases to settle.
7. Drain the lower phase into an appropriate container, and then pour out the upper phase through the neck of the funnel into another container

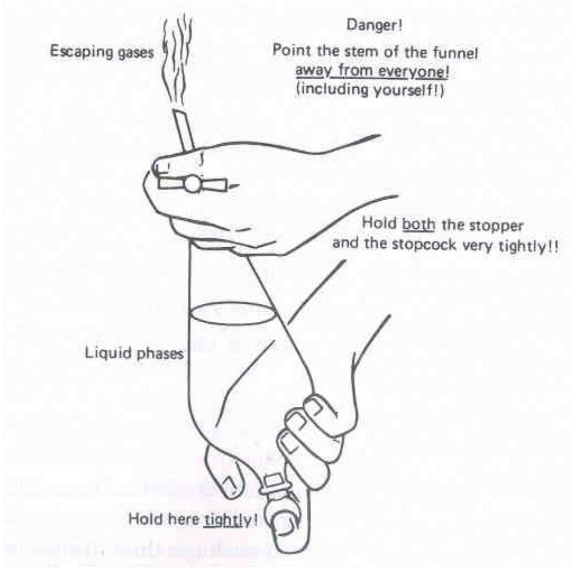




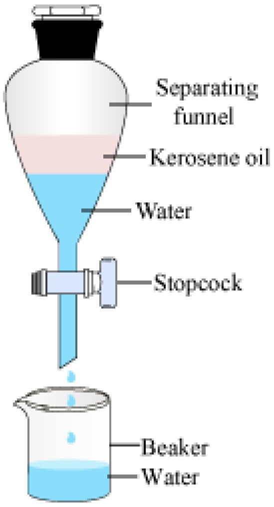
(a) Organic solvent less dense than water (b) Organic solvent more dense vater

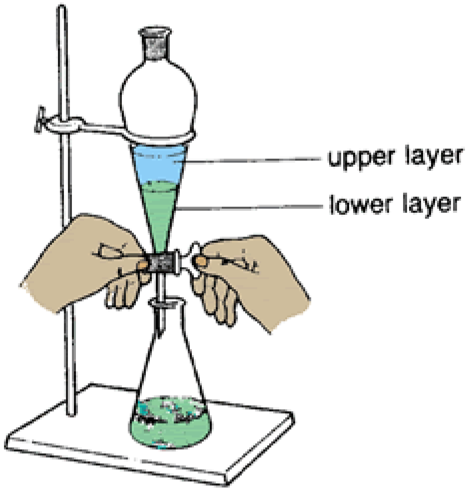


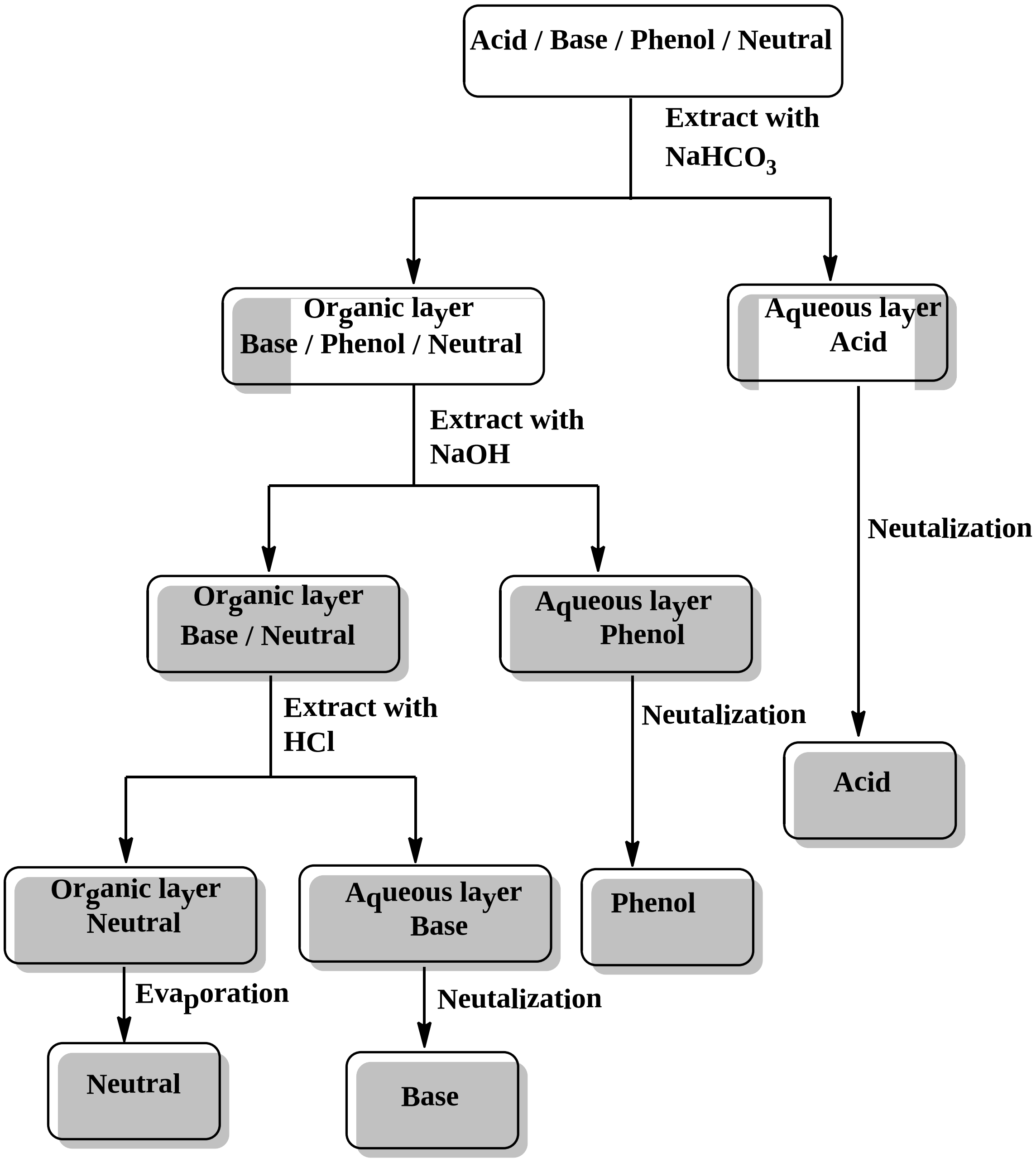
Wrong



Correct






Acid / Base / Phenol / Neutral
Extract with $NaHCO_3$
Organic layer
Base / Phenol / Neutral
Aqueous layer
Acid
Extract with NaOH
Neutalization
Organic layer
Base / Neutral
Aqueous layer
Phenol
Extract with HCl
Neutalization
Acid
Organic layer
Neutral
Aqueous layer
Base
Phenol
Evaporation
Neutalization
Neutral
Base

(Cannizzaro reaction)

Synthesis of benzoic acid and benzyl alcohol

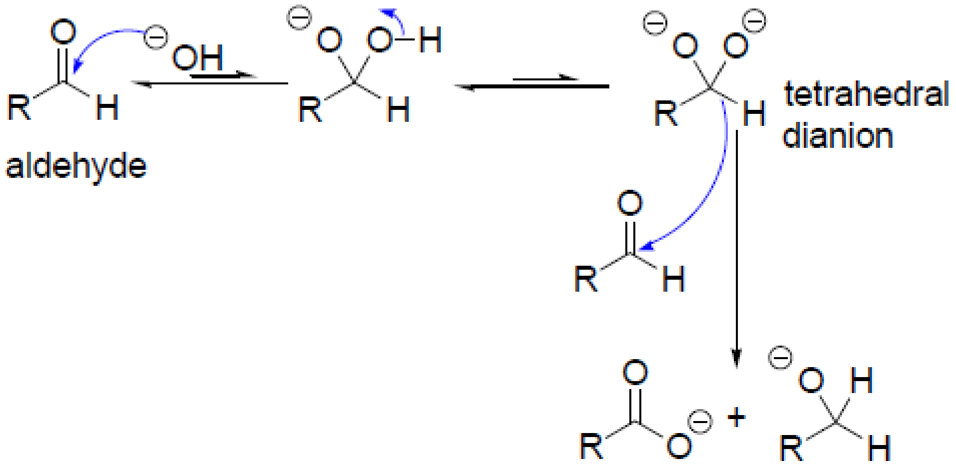
Apparatus and chemicals

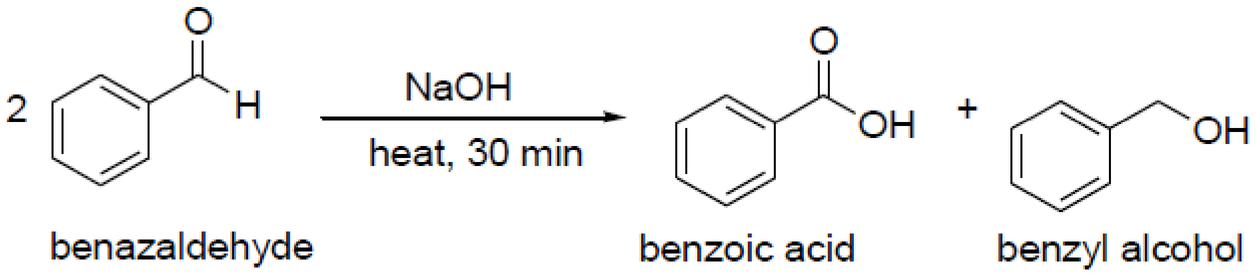
Chemicals	Apparatus
benzaldehyde (7.5 g) sodium hydroxyde (4.5 g) dichloromethane (50 mL) sodium bisulfate solution $NaHSO_3$ (5 mL) anhydrous sodium sulfate (1 g) conc. hydrochloric acid	100 mL beaker, 50 mL beaker bain-marie separating funnel funnel, filter paper Büchner funnel, filter paper, pH paper, ice

Introduction

As a general rule, nucleophilic addition reactions are characteristic only of aldehydes and ketones, not of carboxylic acid derivatives. The reason for the difference of is structural; the tetrahedral intermediate produced by addition of a nucleophile to a carboxylic acid derivative can eliminate a leaving group, leading to a net nucleophilic acyl substitution reaction. The tetrahedral intermediate produced by addition of a nucleophile to an aldehyde or ketone, however, has only alkyl or hydrogen substituents and thus can't usually expel a leaving group. One exception to this rule, however, is the **Cannizaro reaction,** discovered in 1853. The Canizzaro reaction takes place by nucleophilic addition of OH^- to an unenolizable aldehyde (bearing no α H) to give a tetrahedral intermediate, which expels hydride ion as a leaving group and is thereby oxidized. A second aldehyde molecule accepts the hydride ion in another nucleophilic addition step and is thereby reduced.

GENERAL MECHANISM





PROCEDURE

In a 50 mL beaker introduce benzaldehyde (7.5 g) and a saturated solution of sodium hydroxyde (4.5 g of pellets in the minimum amount of water). Heat the mixture on hotplate or water bath for 30 min while stirring vigorously (**Figure 3.1**). Next, cool the beaker down and the minimum amount of cold water to dissolve the solid. Then, transfer the mixture into a separating flask, extract with dichloromethane (2x20 mL) and collect the organic layers in a 100 mL beaker (**Figure 3.2**). The content of the beaker is mixed vigorously with a solution of sodium bisulfate (5 mL) in order to remove the unreacted benzaldehyde. If a precipitate is formed, filter through a Büchner funnel (**Figure 3.3**) and wash it with dichloromethane (10 mL); finally dry it and weigh it out. The organic layer is washed consecutively with a dilute solution of sodium hydroxyde (5 mL) and water until a neutral pH is reached. The resulting organic layer is dried over anhydrous sodium sulfate (~1g) and evaporated by means of a rotary evaporator. **Weigh the mass of product (benzyl alcohol) obtained.** The remaining aqueous phase is cooled down in an ice bath and treated with concentrated HCl until pH = 1. The resulting solid is filtered through a Büchner funnel, washed twice with cold water and finally dried over filter paper.

Recrystallization

Dissolve the solid completely in hot water (30 mL) and let the solution cool down slowly until benzoic acid crystallizes in the form of white needles. The solid is isolated by filtration and dried over filter paper. **Weigh the mass of product (benzoic acid) obtained.**

BOILING POINTS AND DISTILLATION

Distillation is a method for separating two or more liquid compounds by taking advantage of their boiling-point differences. Unlike the liquid-liquid and liquid-solid separation techniques of extraction and crystallization, distillation is a liquid-gas separation in which vapor pressure differences are used to separate different compounds.

A liquid at any temperature exerts a pressure on its environment. This ***vapor pressure*** results from molecules leaving the surface of the liquid to become vapor.

$$\text{Molecules}_{\text{liquid}} \rightleftharpoons \text{molecules}_{\text{vapor}}$$

As a liquid is heated, the kinetic energy of its molecules increases. The equilibrium shifts to the right and more molecules move into the gaseous state, thereby increasing the vapor pressure. shows the relationship between vapor pressure and temperature for pentane, hexane, water, and octane (Fig.1).

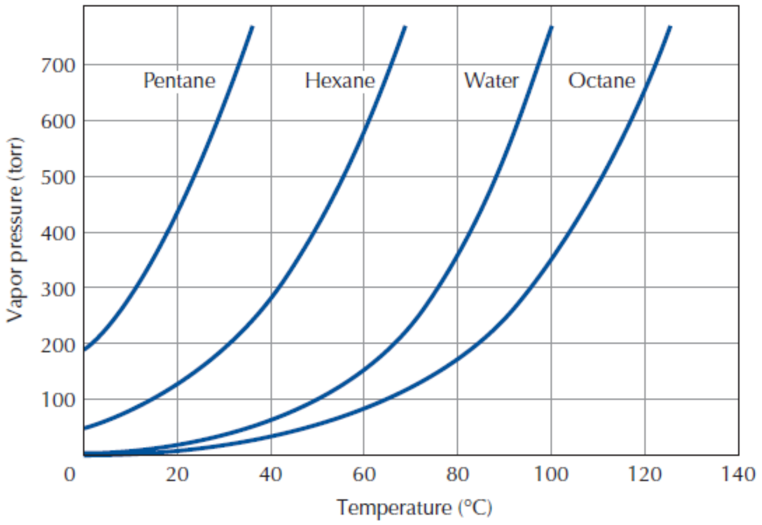


Figure.1. relationship between vapor pressure and temperature for pentane, hexane, water, and octane .

Distillation and Separation of Mixtures

I.1. Distillation Theory

The boiling point of a pure liquid is defined as the temperature at which the vapor pressure of the liquid exactly equals the pressure exerted on it by the atmosphere and is one of its characteristic physical properties. The boiling point is used to characterize a new organic liquid, and knowledge of the boiling point is used to compare one organic liquid with another, as in the process of identifying an unknown organic substance.

The boiling point of a mixture is a function of the vapor pressures of the various components in the mixture. Impurities either raise or lower the observed boiling point of a sample, depending on how the impurity interacts with the compound for which the boiling point is being measured.

Consider, for example, the boiling characteristics of a mixture of pentane and hexane. Pentane and hexane are *miscible* (mutually soluble), and their molecules interact with one another only by weak van der Waals forces. A solution composed of both pentane and hexane will boil at temperatures intermediate between the boiling points of pentane (36 ^{o}C) and hexane (69 ^{o}C). If pentane alone was present, the vapor pressure above the liquid would be due only to pentane. However, with pentane as only a fraction of the solution, the vapor pressure exerted by pentane (P) will be equal to only a fraction of the vapor pressure of pure pentane at the same temperature (P^{o}), where X is the *mole fraction* of pentane, the fraction of pentane molecules in solution. The same is true for the hexane component (Eqs. 1 and 2).

$$P_{\text{hexane}} = P^{o}_{\text{hexane}} X_{\text{hexane}} \qquad \text{(Eq.1)}$$

$$P_{\text{pentane}} = P^{o}_{\text{pentane}} X_{\text{pentane}} \qquad \text{(Eq.2)}$$

Using Dalton's law of partial pressures, we can now calculate the total vapor pressure of the solution, which is the sum of the partial vapor pressures of the individual components.

$$P_{\text{total}} = P_{\text{pentane}} + P_{\text{hexane}} \qquad \text{(Eq.3)}$$

Being able to calculate the total vapor pressure of a solution can be extremely useful to a chemist; knowing the composition of the vapor above a solution can be just as important.

Here is an illustration of how it is done: Applying the ideal gas law to the mixture of gases above a solution of pentane and hexane, we have equation 4. Y is the fraction of pentane molecules in the vapor above the solution.

$$Y_{\text{pentane}} = P_{\text{pentane}}/P_{\text{total}} \qquad \text{(Eq.4)}$$

With the fact that the total mole fractions of pentane and hexane must equal one, a single expression for the total vapor pressure of the solution (equation 5) can be derived.

$$X_{\text{hexane}} + X_{\text{pentane}} = 1$$

$$P_{\text{total}} = X_{\text{pentane}}\,(P^{\text{o}}{}_{\text{pentane}} - P^{\text{o}}{}_{\text{hexane}}) + P^{\text{o}}{}_{\text{hexane}} \qquad \text{(Eq.5)}$$

Finally, the combination of equations 4 and 5, plus Raoult's law, allows the calculation of the mole fraction of pentane in the vapor state.

$$Y_{\text{pentane}} = P^{o}{}_{\text{pentane}}\,X_{\text{pentane}} \,/\, X_{\text{pentane}}\,(P^{o}{}_{\text{pentane}} - P^{o}{}_{\text{hexane}}) + P^{o}{}_{\text{hexane}}$$
(Eq.6)

So, if you know the vapor pressures of pure pentane and pure hexane at various temperatures and the composition of the liquid you can calculate the fraction of pentane in the vapor above the solution. This kind of calculation can be used to construct a temperature-composition diagram, sometimes called a *phase diagram*.

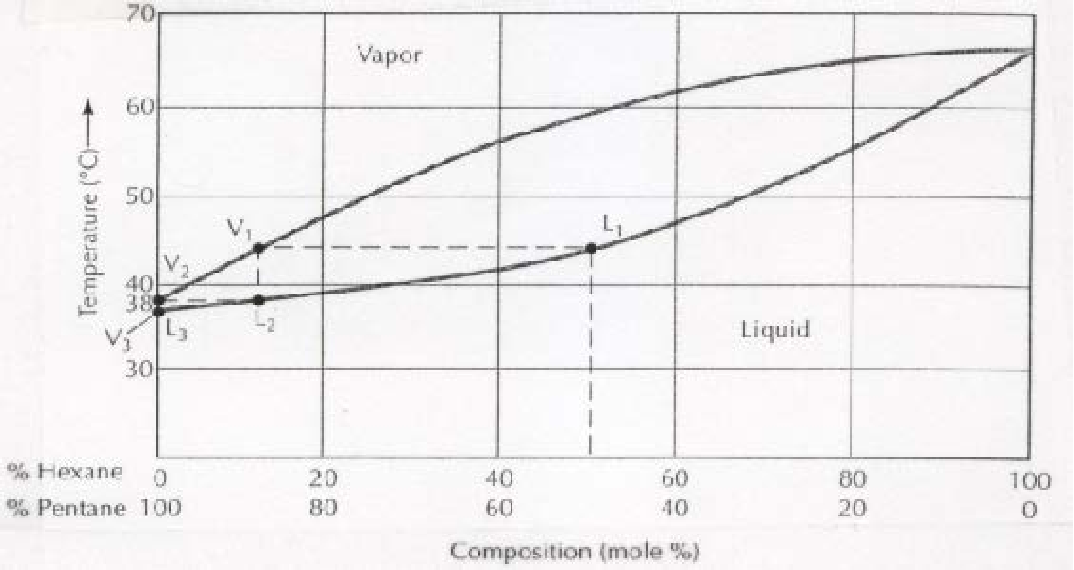


Figure.2. Estimated temperature-composition diagram for pentane-hexane solutions at 1.0 atm pressure.

It is useful to follow the dotted line in, moving from L_1 to V_1 to L_2, etc. Point L_1 indicates a boiling point of 44 °C at atmospheric pressure for a solution containing a 1:1 molar ratio of pentane to hexane. Upon removing a sample of the vapor, we find that it has a molar composition of 87% pentane and 13% hexane as indicated by point V_1. The mole fraction of the component with the lower boiling point is greater in the vapor than in the liquid. If the vapor at V_1 condenses, the liquid that collects (L_2) will have the same composition as the vapor (V_1). Now, if the condensed liquid (L_2) is revaporized, the new vapor will be even richer in pentane (V_2). Repeating the boiling and condensing several more times allow us to obtain pure pentane,uncontaminated by hexane.

As pentane is removed in the vapor, the composition of the liquid, originally L_1, becomes richer in hexane, the component with the higher boiling point. As the mole fraction of hexane in the liquid increases, the boiling point of the liquid also increases until the boiling point of pure hexane, 69 ^{o}C, is reached. In this way pure hexane can also be separated.

In a *simple distillation*, only one vaporization and condensation occurs, corresponding to points L_1 and V_1. This process would not effectively separate a mixture such as pentane and hexane. If a 1:1 solution of pentane and hexane undergoes a simple distillation, the first vapor that condenses has a molar composition of 87% pentane and 13% hexane (figure 3). The molar composition of the remaining liquid now contains more hexane and less pentane than originally; consequently, the boiling point of the mixture will increase. As additional vapor condenses into the receiving flask, the boiling point of the remaining mixture continues to increase. We can represent this graphically by a distillation curve showing vapor temperature versus volume of distillate for the simple distillation of our pentane-hexane mixture.

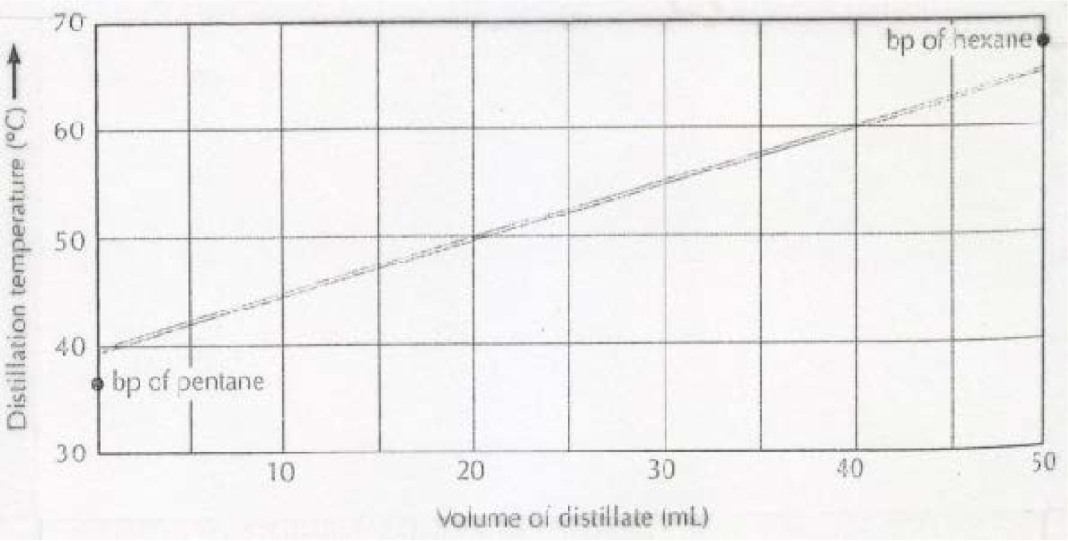


Figure.3. Distillation curve for a simple distillation of a 1:1 molar solution of pentane and hexane.

The initial distillate is collected at a temperature above the boiling point of pure pentane and the final distillate never reaches the boiling point of pure hexane, a result indicating a poor separation of the two compounds. Even though simple distillation does not effectively separate a mixture of liquids whose boiling points differ by less than 60-70 ^{o}C, organic chemists use simple distillations in two commonly encountered situations. The last step in the purification of a liquid compound usually involves a simple distillation to obtain the pure product and determine its boiling point. Simple distillation is also used to remove the solvent when recovering an organic compound with a high boiling from a solution.

I.2. Simple Distillation

In a simple distillation, **the distilling flask should be only one-third to one-half full of the liquid being distilled.** With a flask that is too full, liquid can easily bump over into the condenser. If the flask is nearly empty, a substantial fraction of the material will be needed just to fill the flask and distilling head with vapor. When the desired liquid is dissolved in a large quantity of a solvent with a lower boiling point, the distillation should be interrupted after almost all of the solvent has been distilled and the higher-boiling liquids should be poured into a smaller distilling flask before continuing the distillation.

I.2.1.Miniscale Distillation

The following figure 4 shows the miniscale apparatus for a simple distillation. The assembly of the apparatus is explained in detail in the following steps.

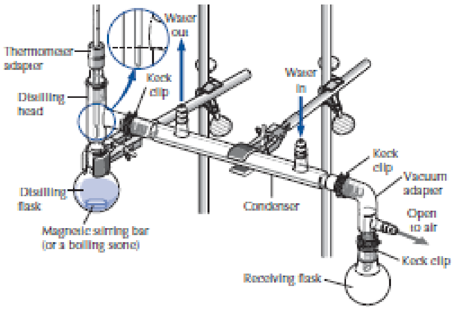


figure 4. Miniscale apparatus for a simple distillation.

1. Select a round-bottomed flask of a size that will be one-third to one-half filled with the liquid being distilled. Place a clamp firmly on the neck of the

flask and attach the clamp to a ring stand or support rod. Using a conical funnel, pour the liquid into the flask. Add one or two boiling stones.

Boiling stones should never be added to a hot liquid because they may cause a superheated liquid to boil violently.

2. *Lightly* grease the bottom joint and the side-arm joint on the distilling head. Fit the distilling head to the round-bottomed flask and twist the joint to achieve a tight seal. Finish assembling the rest of the apparatus before inserting the thermometer adapter and thermometer. **Note: The distilling flask and distilling head need to be in a completely vertical position so that the condenser is positioned with a downward slant.**
3. Attach rubber tubing to the outlets on the condenser jacket. Wire hose clamps are often used to prevent water hoses from being blown off the outlets by a surge in water pressure. Grease the inner joint at the bottom of the condenser, attach the vacuum adapter, and while the pieces are still lying on the desktop, place a Keck clip over the joint.
4. Clamp the condenser to another ring stand or upright support rod, as shown in the previous figure. If the clamp used to support the con- denser has a stationary and a movable jaw, position it with the stationary jaw underneath the condenser and the movable jaw above. Fit the upper joint of the condenser to the distilling head, twist to spread the grease, and place a Keck clip over the joint.
5. Lightly grease the inner joint at the bottom of the vacuum adapter and attach a round-bottomed flask to serve as the receiving vessel. Twist the joint to achieve a tight seal and immediately attach a Keck clip. Although without a clip the receiver flask may stay attached to the vacuum adapter for a time, gravity will soon win out and the flask will fall and per- haps break.

It is usually necessary to have at least two receiving vessels at hand; the first container is for collecting the initial distillate that consists of impurities with *lower boiling points before the expected boiling point of the desired fraction is attained.*

6. Gently push the thermometer through the rubber sleeve on the thermometer adapter. Alternatively, a thermometer with a standard taper fitting may be used instead of the thermometer and rubber-sleeved adapter.
7. Grease the joint on the thermometer adapter and fit it into the top joint of the distilling head. Adjust the position of the thermometer to **align the top of the thermometer bulb with the bottom of the side arm** on the distilling head.
8. Check to ensure that the rubber tubing is tightly attached to the condenser and that **water flows in at the bottom and out at the top. Slowly turn on the water.**
9. Place a heating mantle or other heat source under the distillation flask, using an iron ring or lab jack to support the mantle, and begin heating the flask.

If you use an Erlenmeyer flask or graduated cylinder to collect the distillate, position the outlet of the vacuum adapter slightly inside the mouth of the receiving vessel. A ***beaker should never be used as the receiving vessel*** *because its wide opening readily allows vapors to escape.*

Carrying Out the Distillation

The expected boiling point of the liquid being distilled determines the heat input, controlled by a variable transformer; vaporization of a liquid with a high boiling point requires more heat than does a low-boiling liquid. Heat the liquid slowly to a gentle boil. A ring of condensate will begin to move up the inside of the flask and then up the distilling head. The temperature observed on the thermometer will not rise appreciably until the vapor reaches the thermometer bulb because it is measuring the vapor temperature, not the temperature of the boiling liquid. If the ring of condensate stops rising before it reaches the thermometer, increase the setting on the variable transformer.

When the vapor reaches the thermometer, the temperature reading should increase rapidly. To achieve satisfactory separation of liquids that boil within 100°C of one another, adjust the heat input to maintain a collection rate of 1 drop every 1–2 s. It may be necessary to increase the heat input during the distillation if the rate of collection slows.

Collect any liquid that condenses below the expected boiling point as the first fraction, or forerun which is usually discarded then change to a second receiving vessel to collect the desired fraction when the temperature stabilizes at or slightly below the expected boiling point of the liquid. Record the temperature at which you begin to collect the desired fraction.

As the end of a distillation approaches, it is essential to lower the heat source BEFORE the distillation flask reaches dryness. If the temperature begins to drop, it signifies that vapor is no longer reaching the thermometer bulb and that the distillation should be discontinued. Record the temperature at which the last drop of distillate is collected; the initial and final temperatures are the boiling range of a liquid fraction.

I.2.2. Fractional Distillation

The common use of the term *fractional distillation* refers to a distillation operation in which *a fractionating column* has been inserted between the boiler and the vapor takeoff to the condenser figure 5. The effect of this column is to give in a single distillation a separation equivalent to several successive simple distillations.

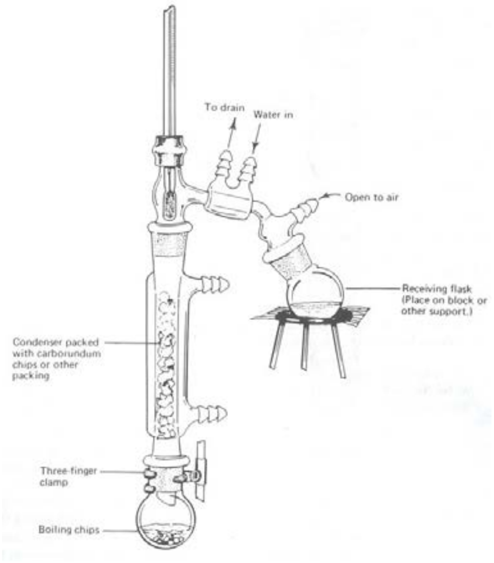


Figure 5. Fractional Distillation Apparatus

In addition to packed columns, special columns are available that achieve; mixing of the ascending vapor and the descending condensate by their special construction. One of the simplest, least expensive, and most widely used is the Vigreux column. It is essentially an empty tube with many finger-like indentations that point downward at a 450 angle. The rising vapors condense on the fingers and any excess liquid drips down to lower parts of the column. The film of condensate on each finger equilibrates with the rising vapor. Under normal working conditions the Vigreux column has a relatively low efficiency (high HETP of 10 cm), but its low resistance to vapor flow permits a large throughput (volume of distillate per unit of time) that makes the column well suited to distillation of bulk solvents. Because of its small surface area the column has a low holdup and is sometimes used for preliminary purification of small samples.

Figure 6. Vigreux Column

I.2.3. Vacuum Distillation

Since the boiling temperature *of* a liquid is decreased by diminishing the pressure on its surface, you can distill at a lower temperature by using an apparatus that is connected to a vacuum pump that maintains a lower inside pressure. This procedure is useful for Purifying liquids (or low- melting solids) that decompose at elevated temperatures.

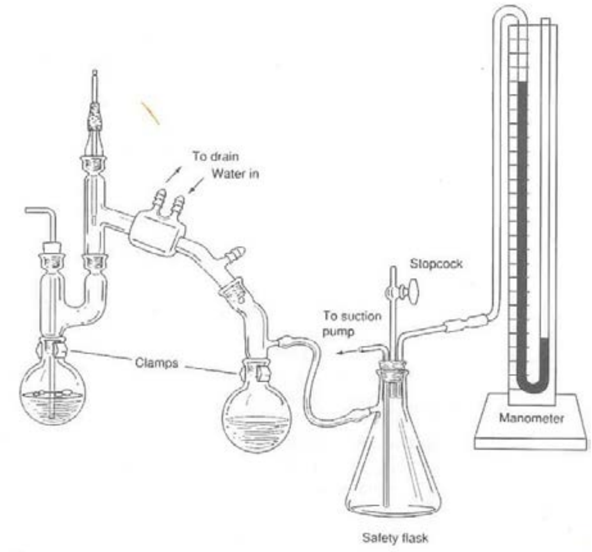


Figure 7. Vacuum Distillation Apparatus

I.2.4. Steam Distillation

Steam distillation consists of distilling a mixture of water and an insoluble or partly soluble substance. The practical advantage of steam distillation is that the mixture usually distills at a temperature below the boiling point of the lower-boiling component. Consequently, it is possible to steam distill a high boiling organic compound at a temperature much below its boiling point (in fact, below 100°) without resorting, to vacuum distillation. Steam distillation is useful also in separating mixtures when one component has an appreciable vapor pressure (at least 5 mm) in the vicinity of 100° and the other has a negligible vapor pressure. The process of steam distillation is widely employed in the laboratory and in industry; e.g., for the isolation of α-pinene, aniline, nitrobenzene, and many natural essences and flavoring oils.

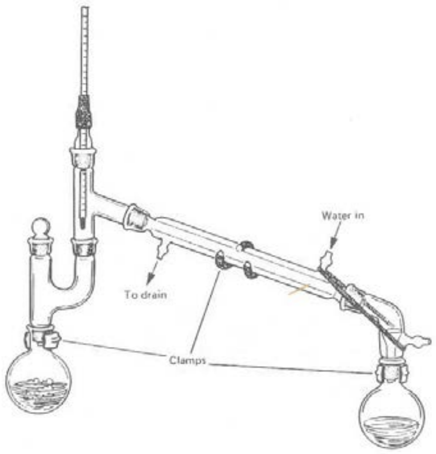


Figure 8. Small-Scale Steam Distillation Assembly

II. Laboratory Practice

The purpose of this section is to provide sufficient practice in purification of liquids by distillation so that this operation can subsequently be carried out skillfully and without reference to detailed directions. Usually only one or two of these procedures will be assigned.

II.1. Simple Distillation

Arrange a distillation assembly similar to the one shown in Figure 4.

II.1.1. Distillation of a Pure Compound

Into the 250-mL boiling flask introduce 100 mL of pure methanol (caution- *flammable liquid)* by means of a clean, dry funnel. Add one or two tiny boiling chips, attach the boiling flask, and make certain that all connections are tight. Place a graduated cylinder beneath the drip tip to serve as receiver. Heat the flask gently until the liquid begins to boil. Adjust the heating rate until the ring of vapor condensation moves up the wall of the flask and past the thermometer into the condenser. Record the temperature when the first few drops of distillate are collected. Continue to distill the liquid slowly (not over 2 mL/min) and record the distilling temperature at regular intervals during the distillation when the total distillate amounts to 1, 2, 3, etc., mL. Discontinue the distillation (and turn off the heat source) when all but 1 mL of the liquid has distilled. Record the temperature range from the beginning to the end of the distillation; this is the observed boiling point. If the boiling point differs from the literature value, record the correction in your laboratory notebook for future reference.

Transfer the used methanol to a bottle provided for this purpose. From your data, draw a distillation graph for pure methanol, plotting distilling temperatures on the vertical axis against total volume of distillate on the horizontal axis.

II.1.2. Fractional Distillation

Arrange an assembly for fractional distillation as shown in Figure 5.

(A) Methanol and Water

For the separation of a 50:50 mixture (by volume) of methanol and water, the following temperature ranges are satisfactory for the fractions: A, 64-70; B, 70-80; C, 80-90; D, 90-95; and E, residue. Plot your data for the distilation temperature versus volume distilled and by selecting the curve closest to your data estimate the number of theoretical plates obtained.

(B) Acetic Acid and Water

In this experiment you will fractionally distill a mixture of glacial acetic acid and water (100: 31.5 by volume, 1: 1 mole ratio) and follow the progress of separation by titrating 0.5-mL portions of several fractions against standardized aqueous sodium hydroxide with phenolphthalein indicator to determine the acetic acid content. The acetic acid content of the original mixture should be determined in the same way before the material is fractionated. If a column having a large number of plates is used, it will be desirable to use larger portions of the early fractions.

Obtain a 35-mL supply of a 1 : 1 molar solution of acetic acid and water. Fill a 50-mL buret with 1.0 *N* sodium hydroxide solution. With the aid of a 0.5-mL or 1.0-mL pipet, place 0.5 mL of the 1 : 1 molar solution of acetic acid and water in a 50-mL Erlenmeyer flask and add 10 mL of water and a few drops of phenolphthalein indicator. Titrate to a slightly pink end point and record the volume of titrant. Repeat the titration on two more 0.5-mL samples of the 1 : 1 molar solution of acetic acid and water and compute the average titer.

Assemble a fractional distillation apparatus using a 50-mL round-bottomed flask for the boiler and a 25-mL graduated cylinder for the receiver.

Place 30 mL of the 1 : 1 mixture in the flask and add a boiling chip. You will need a small test tube that has been marked to show the liquid level when it contains exactly 0.5 mL of liquid.

Heat the mixture until it boils and then adjust the heating rate so that the mixture distills at a *maximum* rate of 1 drop/sec. Note the temperature at which the first drop distills. Collect the first

0.5 mL of distillate in your marked test tube and the next 4.5 mL in the graduated cylinder. Record the distillation temperatures at each 1-mL interval. Transfer the 0.5-mL sample to a 50-mL Erlenmeyer flask (rinse the tube with a total of 10 mL of distilled water and add the rinse to the Erlenmeyer flask). Mark the flask to indicate the sample it contains.

When the volume of distillate reaches 5 mL, collect another 0.5-mL sample in the test tube and transfer it in the same manner to another Erlenmeyer flask. Collect the next 4.5 mL of distillate in the graduated cylinder, recording the distillation temperatures at each 1-mL interval. Repeat this process at 10 mL, 15 mL, 20 mL, and 25 mL of distillate. Titrate the six samples with the sodium hydroxide solution (the early samples will require very little titrant) and calculate the mole fraction of acetic acid present. In the calculations assume that the volumes of acetic acid and water are additive so that the mole fraction in any sample is simply proportional to the titer value obtained for the initial 0.5 mole fraction mixture.

Prepare a plot of boiling point (ordinate) versus the total volume of distillate (abscissa) and a second plot of the mole fraction of acetic acid versus the total volume of distillate.

Chromatography Technique

Principles of Chromatography

The International Union of Pure and Applied Chemistry (IUPAC) defines chromatography as a physical method of separation in which the components to be separated are distributed between two phases, the immobile stationary phase and the mobile phase. The ***mobile phase*** moves in a definite direction and passes over the ***stationary phase.***

The substances being separated are attracted to the stationary phase by intermolecular forces; the stronger the attraction the slower they migrate through the mobile phase. Separation results from the different migration rates. The adsorption-desorption process with the stationary phase occurs many times as a molecule moves through a chromatography column or on a plate. The time required to move through the mobile phase depends mainly on the proportion of time it is adsorbed on the stationary phase and held immobile. The movement of compounds that have stronger intermolecular forces with the stationary phase is retarded in proportion to their interaction.

All chromatographic methods depend on the distribution of the compounds being separated between the mobile and stationary phases**.** A dynamic equilibrium exists between the sample components dissolved in the stationary phase and those dissolved in the mobile phase. The most commonly used polar stationary phase in liquid and thin- layer chromatography is silica gel, finely ground silica (SiO_2) particles that are coated with a thin layer of water molecules. Intermolecular hydrogen bonding and dipole- dipole interactions allow polar organic compounds to be attracted by the water-coated silica gel much more than nonpolar organic compounds. Therefore, polar organic compounds are carried more slowly by the mobile solvent phase through the stationary phase and leave a chromatography column later than nonpolar compounds. In the same manner, polar organic solvents move compounds faster through a chromatography column and on a TLC plate than nonpolar solvents do.

Because the layer of liquid coating on the stationary phase is very thin, much of the interaction takes place near the surface of the liquid. Rather than absorption into the bulk liquid, a process of surface ***adsorption*** onto the stationary phase occurs. Absorption can be compared to eating a pie and adsorption to a pie hitting your face and clinging to it.

When the compounds being separated adsorb onto the liquid coating of the stationary phase, they ***partition*** themselves between the stationary liquid phase and a mobile liquid or gas phase. The partitioning occurs in the same way a solute partitions itself between two immiscible solvents used for an extraction. The compounds being separated adsorb onto and desorbs from a liquid stationary phase many, many times as the solvent passes through. The tighter they adsorb to the stationary phase, the slower they travel through the chromatography column.

Chromatography in the Organic Lab

Three modern chromatographic methods used in organic chemistry are carried out in glass or metal columns:

1• Liquid chromatography (LC), which uses either a gravity flow of solvent through a stationary phase or a modest pressure to force the solvent through the column at a faster rate (flash chromatography). Usually silica gel, which has a thin film of water on its surface, is the stationary phase.

2• High-performance liquid chromatography (HPLC), which uses high-pressure pumps to force the mobile phase through a very small diameter column that contains the stationary phase.

3• Gas-liquid chromatography (GC), where the mobile phase is a stream of an inert gas. Rather than using a column, thin-layer chromatography (TLC) is carried out on small glass, aluminum, or plastic plates covered with a thin coating of silica gel. Capillary action on the thin surface allows the mobile phase to ascend the plate.

In gas-liquid chromatography (GC) the mobile phase is an inert gas such as helium or nitrogen. The stationary phase is a thin film of a nonvolatile liquid. The column is heated and the compounds pass through the chromatography column somewhat in the order of their volatilities, although specific intermolecular forces with the stationary phase also play a role in the separation. In GC, the mobile phase does not interact with the compounds being separated and does not appreciably cause them to desorb from the stationary phase. It simply carries them down the column when they are in the vapor state. In LC, the mobile-phase liquids compete actively with the stationary phase to attract the compounds moving through the column.

I. THIN-LAYER CHROMATOGRAPHY

Thin-layer chromatography (TLC) has become a widely used analytical technique. It is simple, inexpensive, fast, and efficient, and it requires only milligram quantities of material. TLC is especially useful for determining the number of compounds in a mixture, for helping to establish whether or not two compounds are identical, and for following the course of a reaction.

In TLC, glass, metal, or plastic plates are coated with a thin layer of adsorbent, which serves as the ***stationary phase.*** The stationary phase is usually polar—silica gel is most widely used. The ***mobile phase*** is a pure solvent or a mixture of solvents; the appropriate composition of the mobile phase depends on the polarities of the compounds in the mixture being separated. Most nonvolatile solid organic compounds can be analyzed by thin-layer chromatography. However, TLC does not work well for many liquid compounds because their volatility can lead to loss of the sample by evaporation from the TLC plate.

I.1. Overview of TLC Analysis

To carry out a TLC analysis, a small amount of the mixture being separated is dissolved in a suitable solvent and applied or spotted onto the adsorbent near one end of a TLC plate. Then the plate is placed in a closed chamber, with the edge nearest the applied spot immersed in a shallow layer of the mobile phase called the ***developing solvent***. The solvent rises through the stationary phase by capillary action, a process called ***developing the chromatogram.***

As the solvent ascends the plate, the sample is distributed between the mobile phase and the stationary phase. Separation during the development process occurs as a result of many equilibrations taking place between the mobile and stationary phases and the compounds being separated. **The more tightly a compound binds to the adsorbent, the more slowly it moves on the TLC plate**. When silica gel is the stationary phase, the developing solvent moves nonpolar substances up the plate most rapidly. As the chromatogram develops, polar substances travel up the plate slowly or not at all.

The TLC plate is removed from the developing chamber (figure 1) when the ***solvent front*** (leading edge of the solvent) is 1—1.5 cm from the top of the plate. The position of the solvent front is marked immediately, before the solvent evaporates, with a pencil line. The plate is then placed in a hood to dry.

Several methods are available for ***visualizing*** the compounds in the sample. Some compounds are colored and their spots can easily be seen. If the TLC plate is impregnated with a fluorescent indicator, the plate can be visualized by exposure to ultraviolet light. Alternatively, the compounds can be visualized using a reagent that produces colored spots. The developed and visualized plate is then ready for analysis of the chromatogram figure 2.

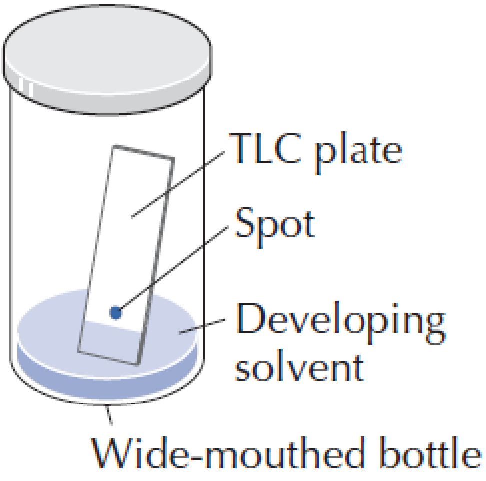
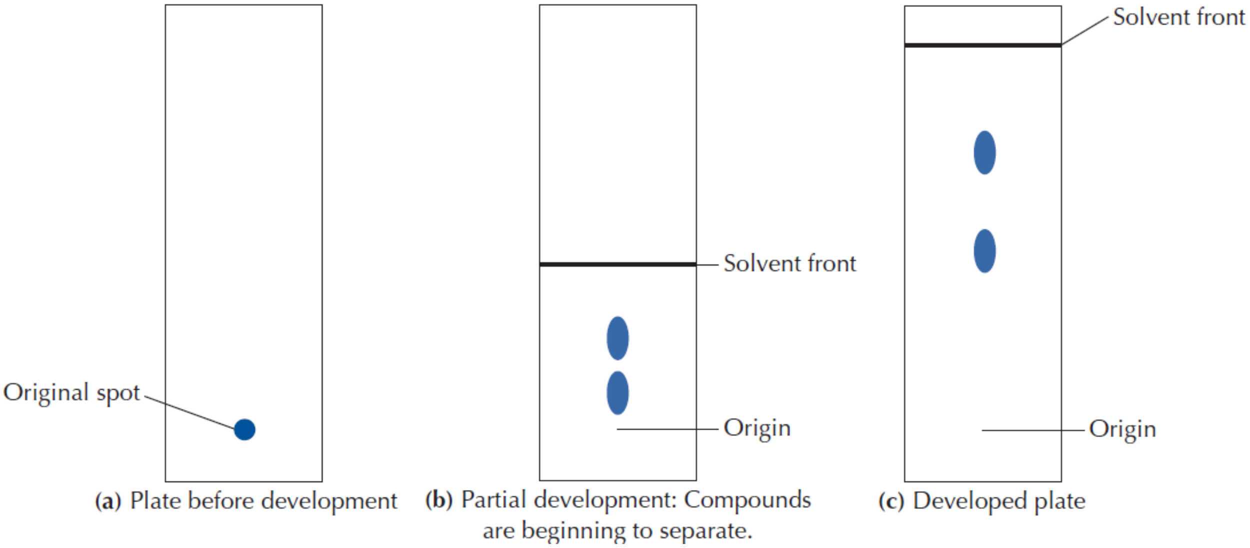


Figure 1. Developing chamber containing a thin-layer plate.



(a) Plate before development **(b)** Partial development: Compounds are beginning to separate. **(c)** Developed plate

Figure 2. Steps in development of a TLC plate

I.2. Plates for Thin-Layer Chromatography

Thin-layer chromatographic plates consist of a solid support, such as glass, metal, or plastic with a thin layer of an adsorbent coating the solid surface, which provides the stationary phase.

I.2.1. Adsorbents

Silica gel ($SiO_2 \cdot xH_2O$) is the most commonly used general-purpose adsorbent for partition chromatography of organic compounds. Aluminum oxide (Al_2O_3, also called alumina) can also be used as a polar adsorbent. Cellulose is used to separate highly polar com- pounds. Several intermolecular forces cause organic molecules to bind to these polar stationary phases. Only weak van der Waals forces bind nonpolar compounds to the adsorbent, but polar molecules can also adsorb by dipole-dipole interactions, hydrogen bonding, and co- ordination to highly polar metal oxide surfaces. The strength of the interaction varies for different compounds, but one generality can be stated: **the more polar the compound, the more strongly it binds to silica gel or alumina.** Another type of silica gel adsorbent used for reverse-phase chromatography has a nonpolar surface that adsorbs less polar compounds more strongly than polar compounds.

I.2.1.1. Silica gel and aluminum oxide

Silica gel and alumina adsorbents are prepared from activated, finely ground powders. Activation usually involves heating the powder to remove some of the adsorbed water. Silica gel is somewhat acidic, and usually it effectively separates acidic and neutral compounds that are not too polar. Aluminum oxide is available in acidic, basic, and neutral formulations for the separation of relatively nonpolar compounds.

If the plastic seal on a package containing precoated silica gel or alumina TLC sheets has been broken for some time, the TLC plates should be activated before use to remove some of the adsorbed water. Activation is done simply by heating the sheets in a clean oven for 15–30 min at the temperature recommended by the manufacturer.

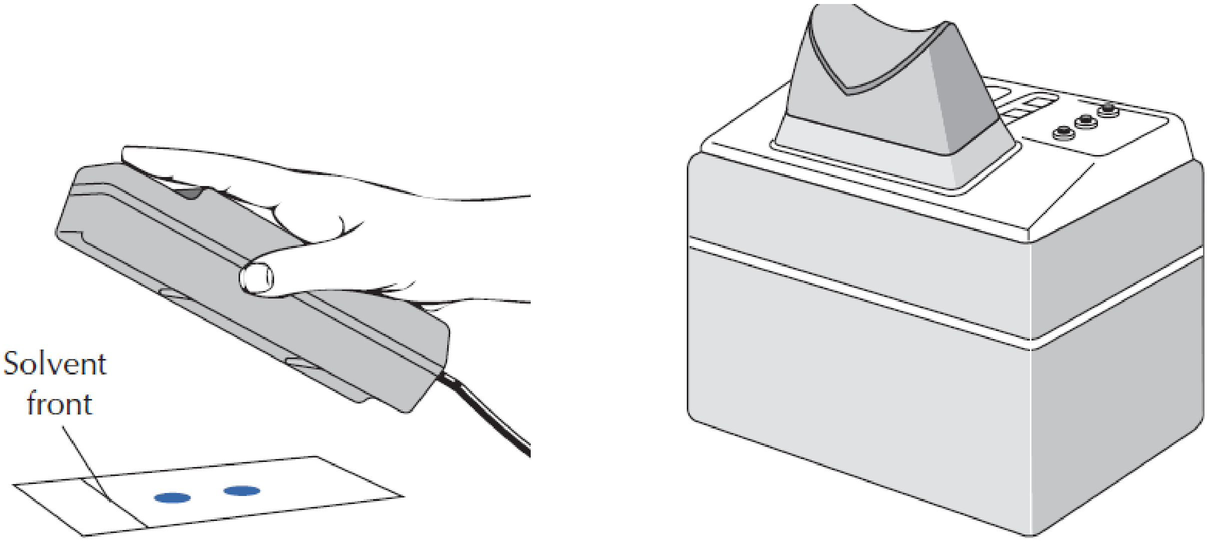
I.2.1.2. Cellulose

Cellulose is less polar than silica gel and alumina and is used for the partition chromatography of water-soluble and quite polar organic compounds, such as sugars, amino acids, and nucleic acid derivatives. Cellulose can adsorb up to 20% of its weight in water; the substances being separated partition themselves between the developing solvent and the water molecules that are hydrogen- bonded to the cellulose particles. Paper chromatography is an example of using cellulose as a stationary phase.

I. 3. Visualization Techniques

Chromatographic separations of colored compounds usually can be seen directly on the TLC plate, but colorless compounds require indirect methods of visualization. Fluorescence and visualization reagents are commonly used to visualize TLC plates. The simplest visualization technique involves the use of adsorbents that contain a fluorescent indicator. The insoluble inorganic indicator rarely interferes in any way with the chromatographic results and makes visualization straightforward. When the output from a short-wavelength ultraviolet lamp (254 nm) is used to illuminate the adsorbent side of the plate in a darkened room or dark box, the plate fluoresces visible light figure 3.

The separated compounds appear as dark spots on the fluorescent field because the substances forming the spots usually quench the fluorescence of the adsorbent, Sometimes substances being analyzed are visible by their own fluorescence, producing a brightly glowing spot. Outline each spot with a pencil while the plate is under the UV source to give a permanent record, which will allow the analysis of your chromatogram.



(a) Using an ultraviolet lamp

(b) Using an ultraviolet lamp with dark box

Figure 3.UV visualization

I.3.1. Visualization Reagents

Not all substances are visible on fluorescent silica gel, so visualization by one of the following methods should also be tried on any unknown sample.

I.3.1.1. Dipping reagents for glass or aluminum plates

Glass or aluminum TLC plates can be dipped briefly in visualizing solutions containing reagents that react to form colored compounds upon heating. Alternatively, the TLC plates can be sprayed with the visualizing solution. Visualization occurs by heating the dipped or sprayed TLC plates with a heat gun or on a hot plate for a few minutes. Three common visualizing solutions are *p*-anisaldehyde, vanillin, and phosphomolybdic acid. The colors fade with time, so the spots should be outlined with a pencil soon after the visualization process.

I.3.1.2. Iodine visualization

Another way to visualize colorless organic compounds uses their absorption of iodine (I_2) vapor. A plastic wash bottle containing a thin layer of iodine crystals is used for this visualization method.

Lay the TLC plate on a clean piece of paper or paper towel. Hold the tip of the wash bottle containing the iodine about 1 cm above the plate and gently squeeze the sides of the bottle as you move it from the bottom to the top of the plate; repeat the motion two or three times. The spots on the plate should appear within 30–60 sec. Yellow-brown colored spots are produced from the reaction of the substances with iodine vapor. If no spots appear, repeat the application of iodine vapors several more times. The colored spots disappear in a short period of time, so they must be outlined with a pencil immediately after they appear. The spots will reappear if the plate is again treated with iodine vapor.

I.4. Analysis of a Thin-Layer Chromatogram

Once the spots on the chromatogram are visualized, you are ready to analyze the chromatogram. The analysis of a thin-layer chromatogram consists of determining the ratio of the distance each compound has traveled on the plate relative to the distance the solvent has traveled.

I.4.1. Determination of the R_f

Under a constant set of experimental conditions, a given compound always travels a fixed distance relative to the distance traveled by the solvent front. This ratio of distances is called the ***R_f* (ratio to the front)** and is expressed as a decimal fraction:

$$R_f = \frac{\text{distance traveled by compound}}{\text{distance traveled by developing solvent front}}$$

The R_f value for a compound depends on its structure as well as the adsorbent and mobile phase used. It is a physical characteristic of the compound, just as its melting point is a physical characteristic. Whenever a chromatogram is done, the R_f value should be calculated for each substance and the experimental conditions recorded. The important data that need to be recorded include the following:

- Brand, type of backing, and adsorbent on the TLC plate
- Developing solvent
- Method used to visualize the compounds
- *Rf* value for each substance

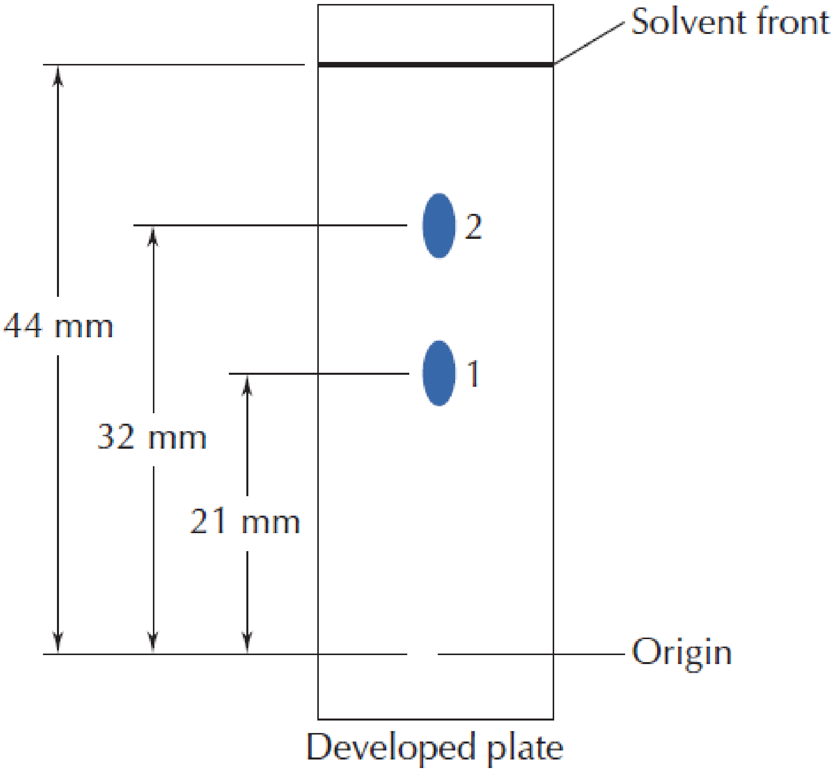


Figure 4. Measurements for the *Rf* value.

I.4.2. Calculation of an Rf Value

To calculate the Rf value for a given compound (figure 4), measure the distance the compound has traveled from where it was originally spotted and the distance the solvent front has traveled from where the compound was spotted. The measurement is made from the center of a spot. The best data are obtained from chromatograms in which the spots are less than 5 mm in diameter. If a spot shows “tailing,” measure from the densest point of the spot. The *Rf* values for the two substances shown on the developed TLC plate in are calculated as follows:

$$\text{Compound 1:}\quad R_f = \frac{21\text{ mm}}{44\text{ mm}} = 0.48$$

$$\text{Compound 2:}\quad R_f = \frac{32\text{ mm}}{44\text{ mm}} = 0.73$$

I.4.3. Identical Rf Values

When two samples have identical *Rf* values, you should not con- clude that they are the same compound without doing further analysis. There are perhaps 100 possible *Rf* values that can be distinguished from one another, whereas there are greater than 10^8 known organic compounds. Further analysis by infrared (IR) or nuclear magnetic resonance (NMR) spectroscopy would be needed to provide definitive evidence about whether the compounds are identical or not. You could conclude that the samples are different com- pounds if subsequent TLC analyses with different developing sol- vents reveal different *Rf* values for each sample.

Summary of TLC Procedure

1. Obtain a precoated TLC plate of the proper size for the developing chamber.
2. Lightly mark the edges of the origin line with a pencil. Spot the plate with a small amount of a 1–2% solution containing the compounds to be separated.
3. Add a filter-paper wick to the developing jar. Then add a suit- able solvent, cap the jar, and shake it briefly to saturate the paper with solvent and the air in the chamber with solvent vapors.
4. Place the spotted TLC plate into the developing jar, taking care that it doesn’t touch the wick, and quickly recap the jar.
5. Develop the chromatogram until the solvent front is 1–1.5 cm from the top of the plate.
6. Mark the solvent front immediately after removing the plate from the developing chamber.

7. Visualize the chromatogram and outline the separated spots.
8. Calculate the *Rf* value for each compound.

How to Choose a Developing Solvent When None Is Specified

Chromatographic behavior is the result of competition by the stationary phase (adsorbent) and the mobile-phase (developing solvent) for the compounds being separated.

A- Solvent considerations. In general, you should use a nonpolar developing solvent for nonpolar compounds and a polar developing solvent for polar compounds. Selecting a suitable solvent is often, however, a trial-and-error process, particularly if a mixture of sol- vents is required to give good separation. A solvent that does not cause any compounds to move from the original spot is not polar enough, whereas a solvent that causes all the spotted material to move with the solvent front is too polar (**Figure 5** a and b). An appropriate solvent for a TLC analysis gives *Rf* values of 0.20–0.70, with ideal values in the range 0.30–0.60, as shown in Figure c.

With a silica gel plate, nonpolar hydrocarbons should be developed with hydrocarbon solvents, but a mixture containing an alcohol and an ester might be developed with a hexane/ethyl acetate mixture. Highly polar solvents are seldom used with silica gel plates, except in the case of reverse-phase TLC.

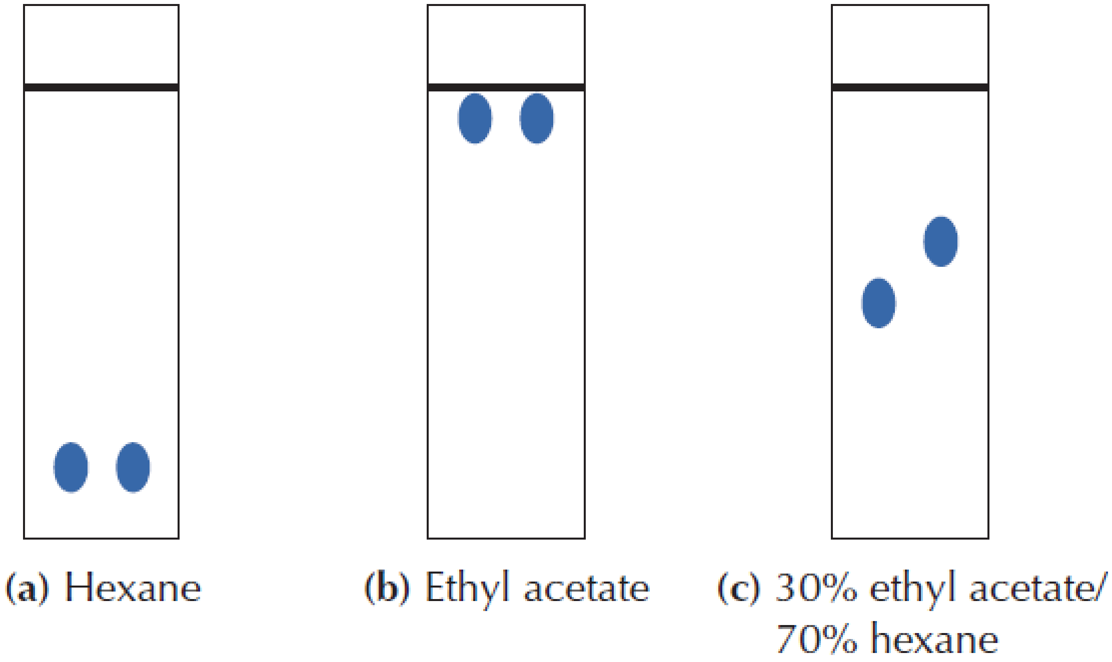
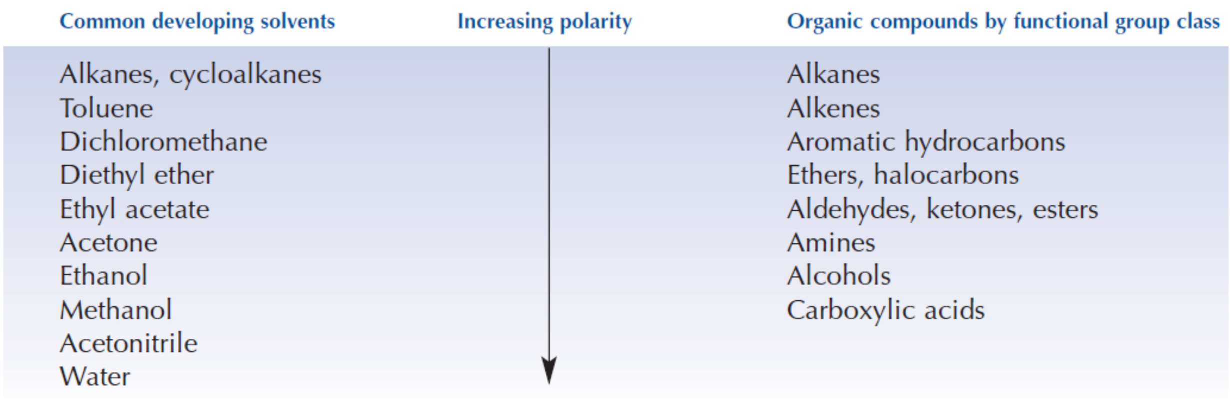


Figure 5. TLC results with different developing solvents.

B. Testing developing solvents. If you know the compounds in the mixture you want to separate, use Table 17.1 to select solvents to test. It shows the relative polarity of common TLC developing solvents and organic compounds by functional group class. If the composition of the mixture is unknown, begin by testing with a nonpolar solvent such as hexane and then with a medium-polarity solvent such as ethyl acetate. When testing mixed solvents, you might start by testing a 50:50 mixture to see how much separation occurs and how far up the plate the two compounds travel. If they travel more than halfway up the plate, test a solvent mixture with a higher percentage of hexane; conversely, if they travel less than halfway up the plate, test a solvent mixture with a higher percentage of ethyl acetate.



Common developing solvents	Increasing polarity	Organic compounds by functional group class
Alkanes, cycloalkanes	↓	Alkanes
Toluene		Alkenes
Dichloromethane		Aromatic hydrocarbons
Diethyl ether		Ethers, halocarbons
Ethyl acetate		Aldehydes, ketones, esters
Acetone		Amines
Ethanol		Alcohols
Methanol		Carboxylic acids
Acetonitrile		
Water		

II. LIQUID CHROMATOGRAPHY

Liquid chromatography (LC), also called column chromatography, and the related methods of flash chromatography and high- performance liquid chromatography (HPLC) are part of the chro- matographic methods so important in experimental organic chemistry. Liquid chromatography is generally used to separate compounds of low volatility, whereas gas chromatography (GC) works only for volatile mixtures. Unlike thin-layer chromatography (TLC) and GC, liquid chromatography can be carried out with a wide range of sample quantities, ranging from a few micrograms for HPLC up to 10 g or more for column chromatography. Most liquid chromatography is carried out under partition conditions.

II.1. Overview of Liquid Chromatography (LC)

In liquid chromatography the stationary phase is a solid adsorbent with a liquid coating, packed into a column. An ***elution solvent*** serves as the mobile phase and consists of either a pure liquid com- pound or a solution of liquids. Gravity draws the elution solvent down the column. Separation occurs by selective interactions of the compounds in the sample with the stationary phase and the mobile phase. The relative polarities of these two phases determine the order in which compounds in the sample elute from the column. Figure 18.1 illustrates how a mixture of two compounds separates on a chromatographic column. With a polar adsorbent such as silica gel, the compound represented by A would be less polar than com- pound B. In reverse-phase chromatography, a relatively nonpolar adsorbent would be used, and the compound represented by A would be more polar than compound B.

II.2. Adsorbents

Most chromatographic separations today use silica gel ($SiO_2 \cdot xH_2O$) because it allows the separation of compounds with a wide range of polarities. Aluminum oxide (alumina, Al_2O_3) is also sometimes used for separations of compounds of low to medium polarity. Silica gel, however, has the advantage of being less likely than alumina to cause a chemical reaction with the substances being separated. Both adsorbents produce a polar stationary phase (aluminum oxide is more polar), and both are generally used with nonpolar to moder- ately polar elution solvents as the mobile phase.

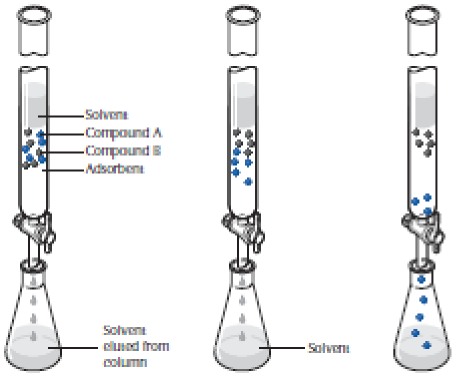
Liquid chromatography at atmospheric or slightly higher pres- sure is used for the purification of samples that require only modest resolution. It uses relatively large greater than 37 µm, adsorbent particles, which allow a reasonably fast flow of the mobile phase under these low-pressure conditions. In HPLC much smaller adsor- bent particles are used, which requires high pressure to force the elu- tion solvent through the column.

II.2.1. Silica Gel

For a simple gravity liquid chromatography column, 63–210 µm (70–230 mesh) particle size silica gel is usually used. Chromatogra- phic silica gel has 10–20% adsorbed water by weight and acts as the solid support for this water under the conditions of partition chromatography. Compounds separate by partitioning themselves between the elution solvent and the water that is strongly adsorbed on the silica surface. The partition equilibria depend on the relative solubilities of the compounds in the two liquid phases. The adsorp- tive properties of silica gel may vary considerably from one manu- facturer to another or even within different lots of the same grade from one manufacturer. Therefore, the solvent system previously used for a particular analysis may not work exactly the same way for another separation of the same sample mixture.

II.2.2. Alumina

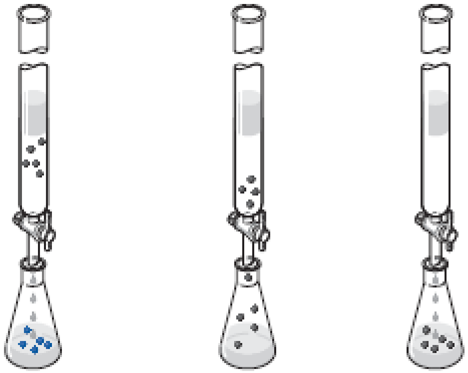
Activated alumina, made explicitly for chromatography, is available commercially as a finely ground powder in neutral (pH 7), basic (pH 10), and acidic (pH 4) grades. Different brands and grades vary enormously in adsorptive properties, mainly because of the amount of water adsorbed on the surface. The strength of the adsorption holding a substance on aluminum oxide depends on the strength of the bonding forces between the substance and the polar surface of the adsorbent.



(a) Mixture of compounds A and B at top of column

(b) Compounds A and B beginning to separate

(c) Compound A starting to elute from column



(d) Compound A collected

(e) Compound B starting to elute from column

(f) Compound B collected

Figure 6. Stages in liquid chromatographic separation of a mixture con taining compound A and compound B. Compound A moves faster than does compound B, which is more strongly adsorbed on the stationary phase.

III. **Separation of Ink Pigments by Thin-Layer Chromatography**

Prepare two 2x 10 cm thin-layer plates by drawing two horizontal pencil lines across each plate 7 mm from each end. On the bottom line of each plate, about 5 mm from the left-hand edge, make a single, sharp dot of ink from a black Flair pen; in the center of the line make a second spot about 2 mm in diameter by momentarily holding the pen tip on the plate; on the right hand side of the line, about 5 mm from the edge, make a third spot about 5 mm in diameter. Add sufficient acetone to an 8-oz, wide-mouth, and screw-cap bottle containing a filter paper lining until a 3 mm deep layer is produced. Center one of the spotted plates in the bottle with the upper edge leaning against the side and screw the cap tightly onto the bottle. When the solvent front reaches the upper pencil line, remove the plate and allow the solvent to evaporate. While the first plate is developing, repeat the process with the other plate and a second 8-oz bottle using a 1 : 1 mixture of acetone and 95% ethanol.

Determine and record the *Rf* values for all of the colored spots. Determine which spots, if any, are UV active. Determine which spots are stained by I_2. Make a sketch of the two plates in your laboratory notebook showing the location and shape of the spots with side notes on their response to UV and I_2.

The experiment can be repeated with other colors of Flair pens to determine if the same dyes are used that were found in the analysis of the pen with black ink.

IV. Separation of Plant Pigments by Thin-Layer Chromatography

In a mortar place 1 g of spinach, 1 g of clean sand, 5 mL of acetone, and 5 mL of mixed "hexanes." Grind the spinach until the green chlorophyll appears to have been extracted completely. Decant the solution into a small beaker.

Prepare two thin-layer plates and in the center of each bottom line place a microdrop of the chlorophyll extract. Blow gently on the spot so that the solvent evaporates quickly. Repeat the addition of the extract several times until a distinct green spot is visible. The additions should superpose as closely as possible.

Develop one plate with 1: 4 (v: v) mixture of acetone and mixed "hexanes". Develop the second plate with a 1: 6: 1 (v: v: v) of acetone, mixed hexanes and etanol 95%.