EXPERIMENTAL PHARMACEUTICAL ORGANIC CHEMISTRY

ASIF HUSAIN

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PREFACE

This book, Experimental Pharmaceutical Organic Chemistry, is meant for D. Pharm and B. Pharm students. The book has been prepared in accordance with the latest syllabi of pharmacy courses. Chemistry is a fascinating branch of science. Practical aspects of chemistry are interesting due to colour reactions, synthesis of drugs, analysis and observation of beautiful crystal development. The important aspects involved in the practicals of pharmaceutical organic chemistry have been comprehensively covered in the book and the subject matter has been organized properly. The language is easy to understand. I hope the students studying pharmaceutical chemistry would be benefitted from this book.

In the book, general and specific safety notes in detail are provided followed by explanation of common laboratory techniques like glassware handling, heating process, crystallization, filtration, drying, melting & boiling point, chromatography etc. A number of equipments, apparatuses and glass wares used in a pharmaceutical chemistry lab are also provided with diagrams. Specific qualitative methods for estimation of elements, functional groups and some individual compounds have been described. Derivative preparation of some organic compounds is presented to further confirm the presence of a particular compound. Syntheses of different organic and pharmaceutical compounds with chemical reaction have also been given.

It is my belief that this book will cater to the needs of the Diploma and undergraduate pharmacy students during their study as well as after completion of their course. Constructive comments on the content and approach of the book from the readers will be highly appreciated. My email address is drasifhusain@yahoo.com.

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ABOUT THE AUTHOR

Dr. Asif Husain is Senior Assistant Professor at the Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi. He received his M.Pharm. and Ph.D. degrees in 1996 and 2000, respectively, from Hamdard University, New Delhi and has been involved in teaching and research for more than 15 years. He has more than 175 peer-reviewed research publications to his credit. Dr. Husain has attended several national and international conferences in India and abroad including USA. He is a recipient of several awards and honors including a visiting fellowship from Youngstown State University, Ohio, USA, and his research has been funded by UGC, AICTE, DST and AYUSH. He has collaboration with different research organizations like National Institute of Health (NIH), National Cancer Institute (NCI), The National Institute of Allergy and Infectious Diseases (NIAID), USA, etc. He has guided a number of M. Pharm/Ph.D. students and authored several books in the field of pharmacy.

ACKNOWLEDGEMENT

"Teacheth man that which he knew not" (Al-Quran)

First of all, I bow in reverence of Almighty Allah, the creator of this universe.

I would like to express my deep sense of gratitude to my parents- my Ammi (mother) Hajjan Shahjahan Begum and my father (Papa) Haji Rafiq Husain sahib, for their constant encouragement, help, love, moral support and prayers. I am highly indebted to my beloved son Ayaan for continuous moral support and keeping my spirits high.

Special thanks and love to my brother Aftab for his help, encouragement and respect. Love showered upon me by Mysha, Arhaan and Arshaan is priceless.

It gives me great pleasure to extend my respect and profound gratitude to my mentor, Late Prof. M.S.Y. Khan Sir, Jamia Hamdard for his help, guidance, motivation and everlasting inspirations. I am highly thankful to my friend Dr Mohammad Shaharyar for his valuable suggestions, help and support. Sincere thanks are also due to my colleagues Dr M. Mumtaz Alam and Dr. M. S. Zaman for their support and help.

Special thanks are due to my student Mohd Arif who has put in great efforts in materializing this work through his inputs. I am thankful to my students Farah Iram and Md Azhar Iqbal for their help in this endeavour.

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1. INTRODUCTION

Chemistry is backbone of pharmacy. Chemistry is considered both basic and applied science. When teaching chemistry, teachers should emphasize both theories and experiments; chemistry experiments play an important role in teaching and serve as an ideal tool for combining theory and practice. Therefore, chemistry experiments should focus on learning goals and developing students' laboratory skills, scientific reasoning skills, knowledge about experimental design, and comprehensive ability. Pharmaceutical Organic chemistry is the branch of chemistry in which covalent carbon compounds and their reactions are studied. A wide variety of classes of compounds such as drugs, vitamins, insulin, natural and synthetic fibres, as well as carbohydrates, peptides, and fats consist of organic molecules. Organic chemists determine the structures of organic molecules, study their various reactions, and develop procedures for the synthesis of organic substances. Organic chemistry is the study of the properties of the compounds of carbon. All carbon compounds except for a few inorganic carbon compounds are organic. Inorganic carbon compounds include the oxides of carbon, the bicarbonates and carbonates of metal ions, the metal cyanides, etc. Organic chemistry is the most important branch of chemistry, but obviously it would be nothing without the other areas of chemistry — in fact all branches of chemistry including pharmaceutical chemistry should not be viewed in isolation, even though they may often be taught in isolation.

Organic chemistry is all around us, life is based on organic chemistry, the food we take, the clothes we wear, the drugs we take, the cars we drive and the fuel that propels them, wood, paper, plastics and paints, etc. Organic chemistry is the study of compounds containing carbon the ability of carbon to form as many as 4 strong bonds to many other atoms, e.g., carbon, hydrogen, oxygen, nitrogen, halogens, sulphur, phosphorus ensures a virtual infinite number of possible compounds the constituent atoms and their exact combination determines the chemical and physical properties of compounds and thus, their suitability for applications. To get understanding of life, we should first understand some fundamentals of organic chemistry. Organic molecules contain both carbon and hydrogen. Though many organic chemicals also contain other elements, it is the carbon-hydrogen bond that defines them as organic.

Pharmaceutical Organic chemistry is very important in life. It affects life; just as there are millions of different types of living organisms on this planet, there are millions of different organic molecules, each with different chemical and physical properties. There are organic

compounds that make up our hair, skin, fingernails, and so on. The diversity of organic chemicals is due to the versatility of the carbon atom. Why is carbon such a special element? Let's look at its chemistry in a little more detail. Carbon (C) appears in the second row of the periodic table and has four bonding electrons in its valence shell. Similar to other non-metals, carbon needs eight electrons to satisfy its valence shell. Carbon, thus, forms four bonds with other atoms, and each bond consisting of one of carbon's electrons and one of the bonding atom's electrons.

Organic chemicals get their diversity from many different ways carbon can bond to other atoms. The simplest organic chemicals, called hydrocarbons, contain only carbon and hydrogen atoms; the simplest hydrocarbon, methane, contains a single carbon atom bonded to four hydrogen atoms. But carbon can bond to other carbon atoms in addition to hydrogen. In fact, the uniqueness of carbon comes from the fact that it can bond to itself in many different ways. Carbon atoms can form long chains. They appear to be almost no limit to the number of different structures that carbon can produce. To add to the complexity of organic chemistry, neighbouring carbon atoms can also form double and triple bonds in addition to single carbon-carbon bonds.

An overall understanding of all the complexity/processes/events/properties/behaviour of atoms/molecules/chemicals helps scientists to develop drugs/compounds/agents which could alleviate the sufferings or make the life better on the earth. Scientists are continuously working to unfold the nature with an aim of betterment of human beings.

2. SAFETY AND PRECAUTIONARY NOTES

Students should keep in mind the following points while working in a chemistry laboratory-

1- Many organic alcohols, phenols, and ethers are toxic, and all are flammable. Acetone is highly flammable.
2- Use these chemicals only in well-ventilated space. Keep away from flames and other sources of ignition.
3- *Sodium* hydroxide is corrosive and can cause burns. Use great care to avoid contact with skin, eyes, and clothing. In case of accidental contact, flood the affected area with copious amounts of water. Spills should be diluted with water and cleaned up immediately.
4- Bromine is corrosive and causes serious burns. Use great care *to* avoid contact with skin, eyes, and clothing. In case of accidental contact, flood the affected area with copious amounts of water and seek medical attention.
5- Chromium is highly toxic and the acid solution is extremely corrosive. Avoid ingestion. Handle only with gloves. Use great care *to* avoid contact with skin, eyes, and clothing. In case of accidental contact, flood the affected area with copious amounts of water. In case of ingestion, seek medical attention immediately.
6- The zinc chloride/hydrogen chloride solution is corrosive and causes burns. Use great care *to* avoid contact with skin, eyes, and clothing. In case of accidental contact, flood the affected area with copious amounts of water. Spills should be diluted with water and cleaned up immediately.
7- Smoking is not allowed in the laboratory. Know the location of fire extinguishers and how to use them.
8- Report all accidents immediately to the instructor.
9- If any person has hair or clothing on fire, as a first step, lie down on the floor and use a blanket, coat or anything available to smother the flames. Get help immediately.
10- Experiments should never be left unattended.
11- Never taste any solid or liquid chemical. When smelling a substance do not hold your face directly over the container.
12- Most organic substances are hazardous to health; so avoid breathing and skin contact as much as possible.
13- Working with toxic, lachrymatory or irritating chemicals must be conducted in fume hoods.
14- In some cases a trap must be used to prevent hazardous gases from escaping into the laboratory atmosphere.
15- If acids or corrosive chemicals are spilled on your skin, wash with plenty of cold water then consult your instructor.
16- Do not point your test tube at your neighbor or yourself when heating substances.
17- Most organic solvents are flammable, so never heat a flammable substance with a direct flame. A hot water bath is used instead.
18- Always wear a laboratory coat. Do not use mobile phone in the laboratory.
19- If acid or base is spilled on your clothing, bench or floor wash thoroughly with water, then neutralize with dilute ammonium hydroxide or acetic acid respectively and inform your instructor.
20- It is advisable to wear safety glasses in the laboratory.
21- Always wash your hands with soap and water on leaving the laboratory.
22- Elemental Na reacts violently and exothermically with water or Oxygen, producing strongly corrosive NaOH and H_2 gas. The latter can ignite spontaneously in this exothermic reaction. Therefore, never leave unprotected Na anywhere and avoid allowing it to come in contact with water.

3. SOME COMMON LABORATORY TECHNIQUES

There are number of laboratory techniques. Some common laboratory procedures are given below which students must be aware of:

GLASSWARE HANDLING

Dirty glassware may be cleaned with soap and water using a brush. However, glassware which has persistent stains from organic substances requires soaking in chromic acid cleaning solution. This mixture has to be used carefully as it is very corrosive. Glass tubing with unpolished ends is a hazard since it can cause serious cuts when trying to insert it into a cork. Therefore, only glass tubing with polished ends must be used. When forcing glass tubing into a cork, grasp it as close as possible to the cork and be careful not to break it. Quickfit glass joints should always be lubricated with a suitable lubricant (grease). A thin film of grease is applied to the joints to provide an air-tight seal and to prevent the joints from being stuck together. There should be no excess grease extending inside the apparatus as it might contaminate the reaction mixture. It is also recommended that old grease be wiped off with a piece of tissue paper before applying a new film.

HEATING DEVICES

There are various heating devices in the laboratory. The Bunsen burner and water bath are the most commonly used. A limitation of the Bunsen burner is that it should not be used directly for heating flammable solvents. Flammable and volatile liquids are heated in a water bath when temperatures under 100 are required. If an electrical steam bath is not available, a large beaker filled with water may be used instead. It is heated to boiling with a Bunsen burner and the flame extinguished before heating the flammable liquid in the bath. Bumping may be prevented by continuous stirring to ensure homogenous and steady heating of the liquid or by the use of boiling stones which achieve a similar effect through formation of bubbles.

REFLUXING

The technique of refluxing is commonly used when it is necessary to heat a reaction in order to bring it to completion in a reasonable time span. A reflux condenser is used to minimize loss, through evaporation, of volatile reactants, products or solvent by allowing the vapors to recondense and return to the reaction vessel

CRYSTALLIZATION AND RECRYSTALLIZATION

The purpose of crystallization and recrystallization is to get pure compound. Crystallization may be defined as the process in which a solid compound precipitates from a saturated solution in the form of crystals. Saturation is usually effected through cooling or evaporation. In certain cases, recrystallization may be used for the separation of a solid mixture. When the impure solid is dissolved in a minimum volume of a suitable hot solvent and the resulting solution is gradually cooled, saturation and eventual crystallization of the pure compound occurs. Impurities in a solid are of two kinds: soluble and insoluble and recrystallization involves the removal of both to purify a solid. Insoluble impurities are first removed by gravity filtration of the hot solution while the soluble impurities remain dissolved in the cold saturated solution (*mother liquor*) after precipitation of the desired compound. The pure crystals are separated from the supernatant liquid by suction filtration. After drying, the purity is checked by a melting point determination.

FILTRATION

Filtration is used whenever an insoluble solid is to be separated from a liquid. Simple gravity filtration (usually hot filtration) is employed to remove insoluble solid impurities from a liquid, while suction filtration (usually cold filtration) is used to collect a desired solid or crystalline product. Decolorization is the removal of colored impurities from a solution. This is achieved by the addition of activated charcoal to the solution and mixing thoroughly. If charcoal is added to a cold solution, the solution is first brought to a boil before hot filtration. When however it is added to a hot solution, the flask should be removed from the heat source before the addition, otherwise bumping will occur. Charcoal is finally removed by filtration leaving an almost colorless solution.

DRYING

The process of drying, if applied to a solid substance is aimed to remove residual solvent (organic or water) adhering to the solid particles/ crystals. This is usually done by air drying (spreading over a sheet of paper/filter paper) and/or heating in an oven to enhance evaporation of the solvent. Drying of an organic liquid, however, involves the removal of traces of water (moisture) using chemical drying agents. Such cases are encountered in extraction where the organic phase is in direct contact with the aqueous phase. After separating the layers, traces of water in the organic phase are removed by the addition of a suitable drying agent. Some common examples are: calcium chloride, magnesium sulfate, sodium sulfate, sodium hydroxide and potassium hydroxide.

MELTING POINT DETERMINATION

The melting point of a solid is the temperature at which transition from solid to liquid occurs at atmospheric pressure; or the temperature at which solid and liquid phases are in equilibrium at a pressure of one atmosphere. A simple device for determining melting points is used and it consists of a thermometer fitted through a cork and suspended into a long-necked flask which is three quarters filled with a high boiling and stable liquid like paraffin oil, di-butylphthalate or silicon oil. The thermometer bulb should be about 1 cm above the bottom of the flask. The sample in the capillary tube is fastened to the thermometer with a rubber band placed above the level of the oil. The capillary tube should be close to and on a level with the thermometer bulb. To determine the melting point of a solid, a small amount of the powdered substance is introduced into a capillary tube which is then attached to a thermometer and placed in the oil bath. The bath is heated rapidly to within 20 ºC of the expected melting point then slowly, and at a constant rate of 2-3 degrees per minute, close to the melting point. The temperature at which the solid begins to melt, and that at which it is completely liquid, is recorded as the melting point range of that substance.

BOILING POINT AND DISTILLATION

The boiling point of a liquid is defined as the temperature at which the vapor pressure of the liquid equals the external pressure (usually 1 atmosphere). It is also defined as the temperature at which vapor and liquid are in equilibrium at a given pressure. The boiling point, like the melting point, is a physical constant and may be used to identify unknown organic liquids. Distillation is the process of heating a liquid to its boiling point, condensing the vapor by cooling, and collecting the liquid distillate. It is a technique for the purification of liquids and for the separation of liquid mixtures. As the distillation progresses, the mixture will gradually have less of the more volatile component and its boiling point will gradually rise. Consequently the distillate will contain a continually decreasing proportion of the more volatile component until finally all has been collected and the less volatile component is left as a residue.

In practice, separation of a liquid mixture into its components by a single distillation (simple distillation) is possible only when the boiling points of the components are 80 degrees or more apart. For mixtures of liquids having boiling points much less than 80 degrees apart, separation can be achieved only by fractional distillation. Such a distillation is equivalent to several repeated simple distillations. It uses a fractionating column which provides a large surface area

for continuous heat exchange between the hot ascending vapor and the cooler descending liquid, thus resulting in a series of evaporations and condensations leading to separation of the two components. Vacuum distillation is a technique for the distillation of high boiling liquids, and for compounds that decompose at atmospheric pressure. At the low pressures employed, those compounds distil at much lower temperatures.

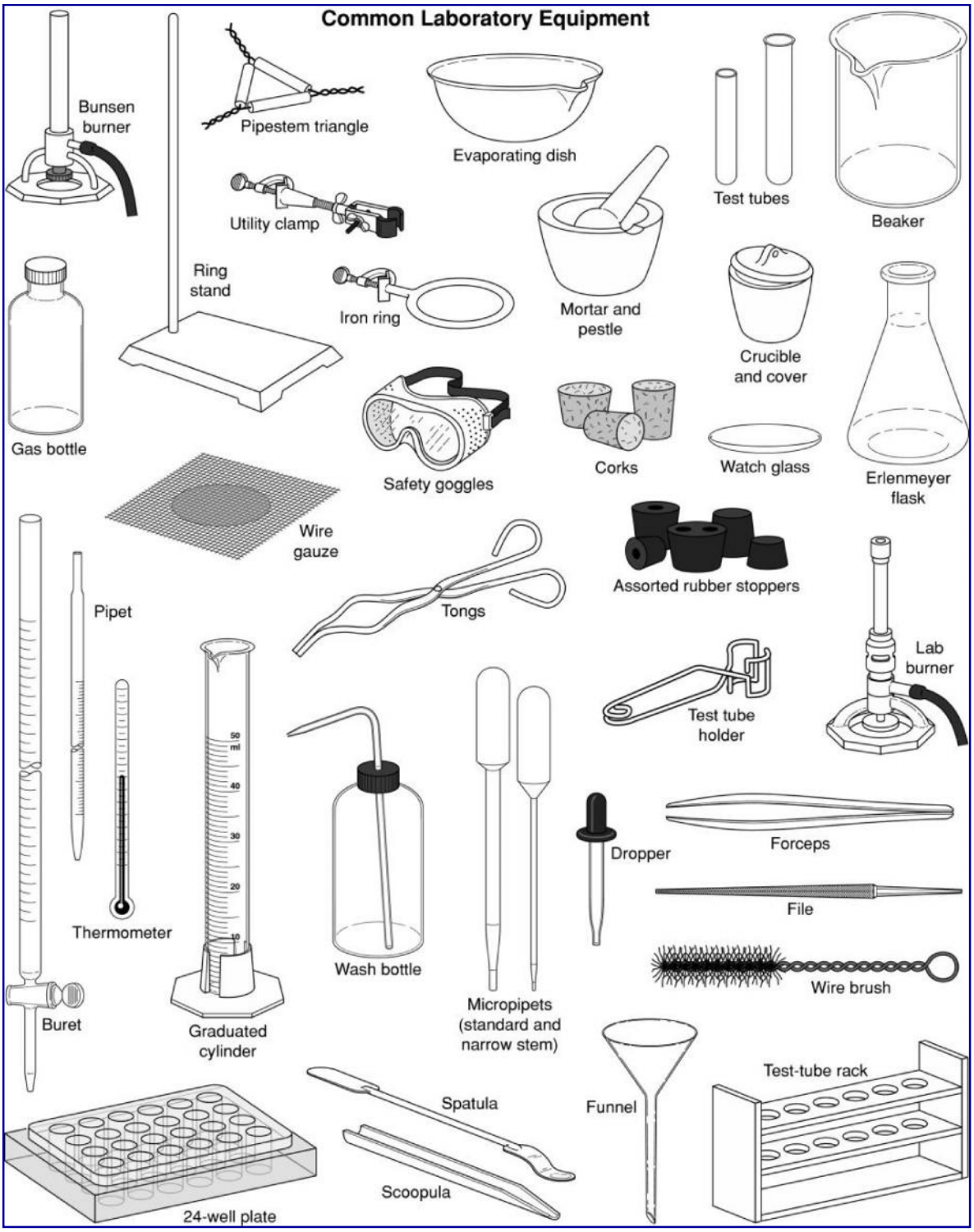
EXTRACTION

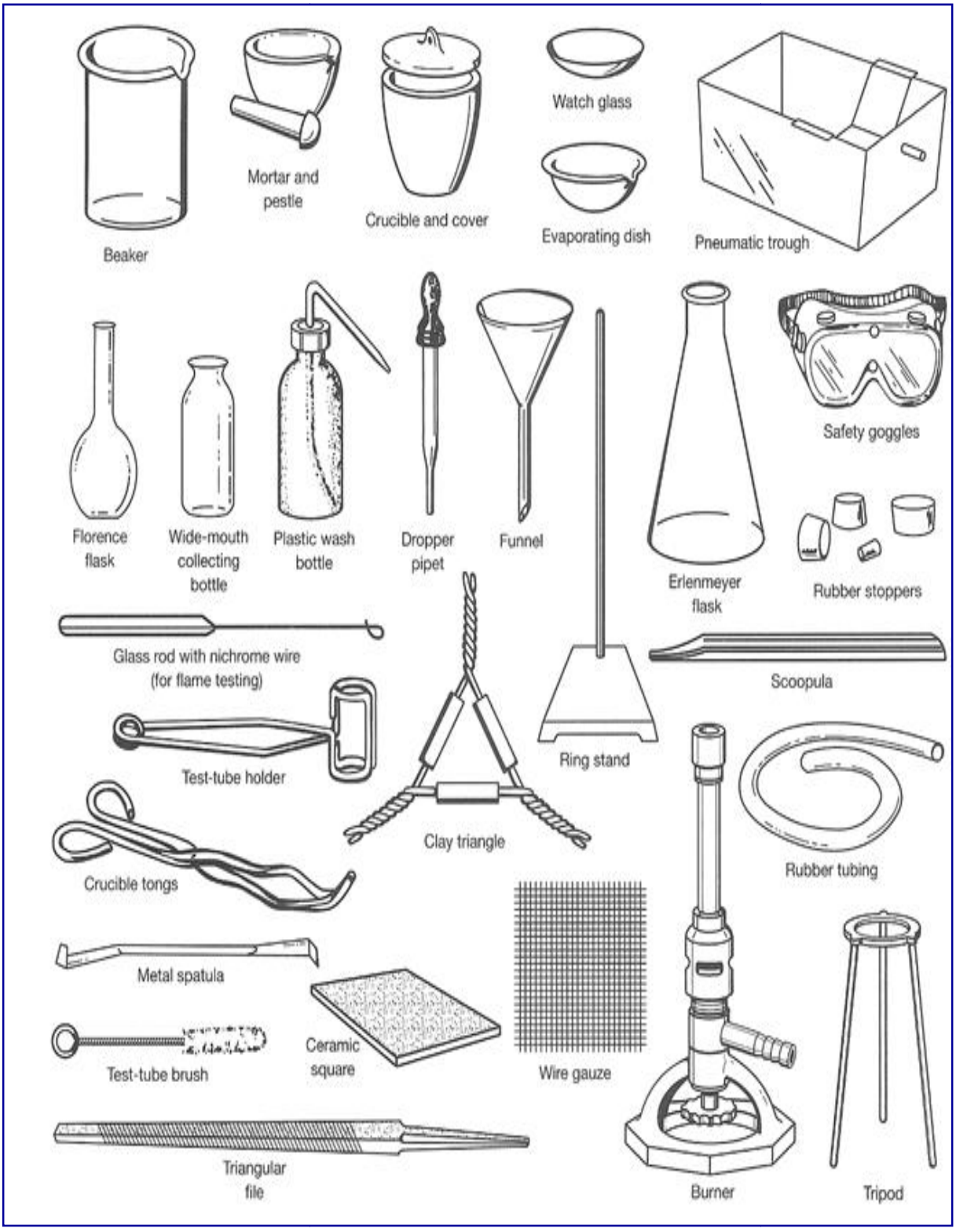
Extraction is the separation of a substance from a mixture by means of a solvent that preferentially dissolves that substance. If the substance is extracted from a solid phase, the process is called solid-liquid extraction, as in the isolation of caffeine from tea leaves by means of hot water. Extraction of a substance from a liquid phase is called liquid-liquid extraction. The most common applications of this latter technique are: (i) The recovery of an organic product from a reaction mixture containing excess unreacted materials and by-products. (ii) Isolation of an organic substance from its natural source, such as a plant.

CHROMATOGRAPHY

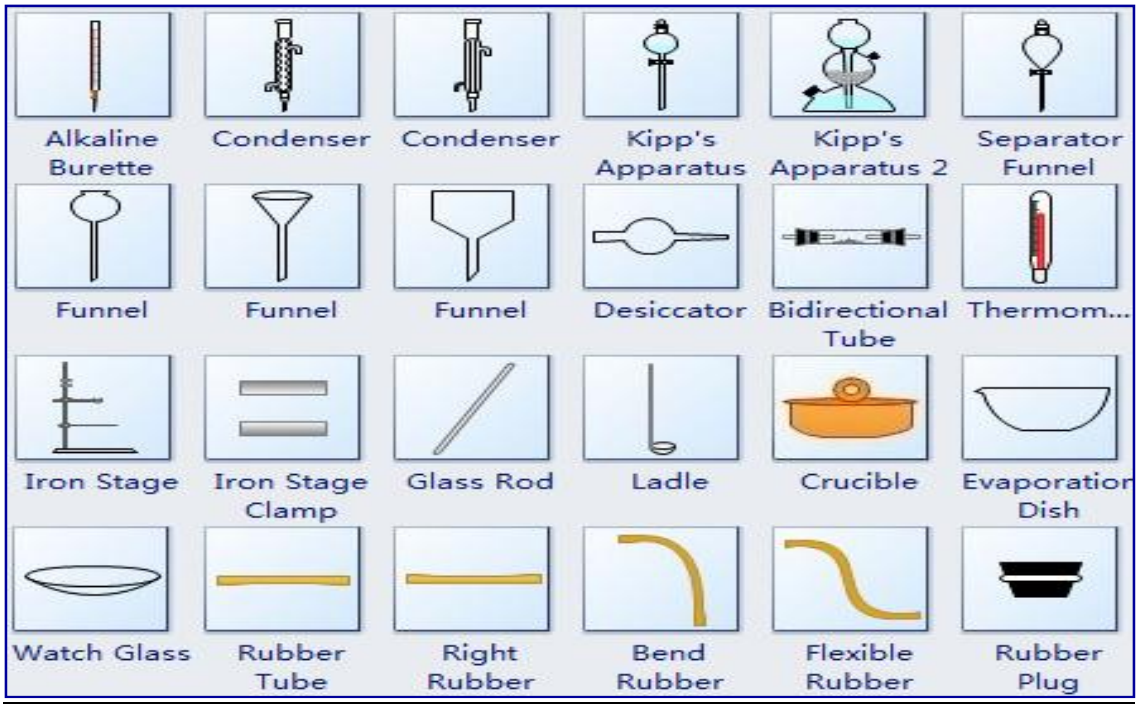
Chromatography is a technique that may be used to separate the components of a mixture as well as to identify organic substances and examine their purity. Chromatography encompasses several techniques such as column, thin-layer, paper, gas liquid, etc. chromatography. Two principles are basically involved in chromatography: adsorption (as in thin layer chromatography) and partition (as in paper chromatography), and certain terms are common to both types of chromatography. In adsorption chromatography, separation depends on the selective desorption of the components of a mixture by the eluent (mobile phase) from the surface of a solid adsorbent (stationary phase). The adsorbent may be packed in a column (column chromatography) or spread as a thin layer on a glass plate as in thin-layer chromatography. In partition chromatography, separation depends on partition of the components of a mixture between the stationary and mobile phases. The mobile phase may be a liquid (liquid-liquid partition chromatography) or a gas (gas-liquid partition chromatography).

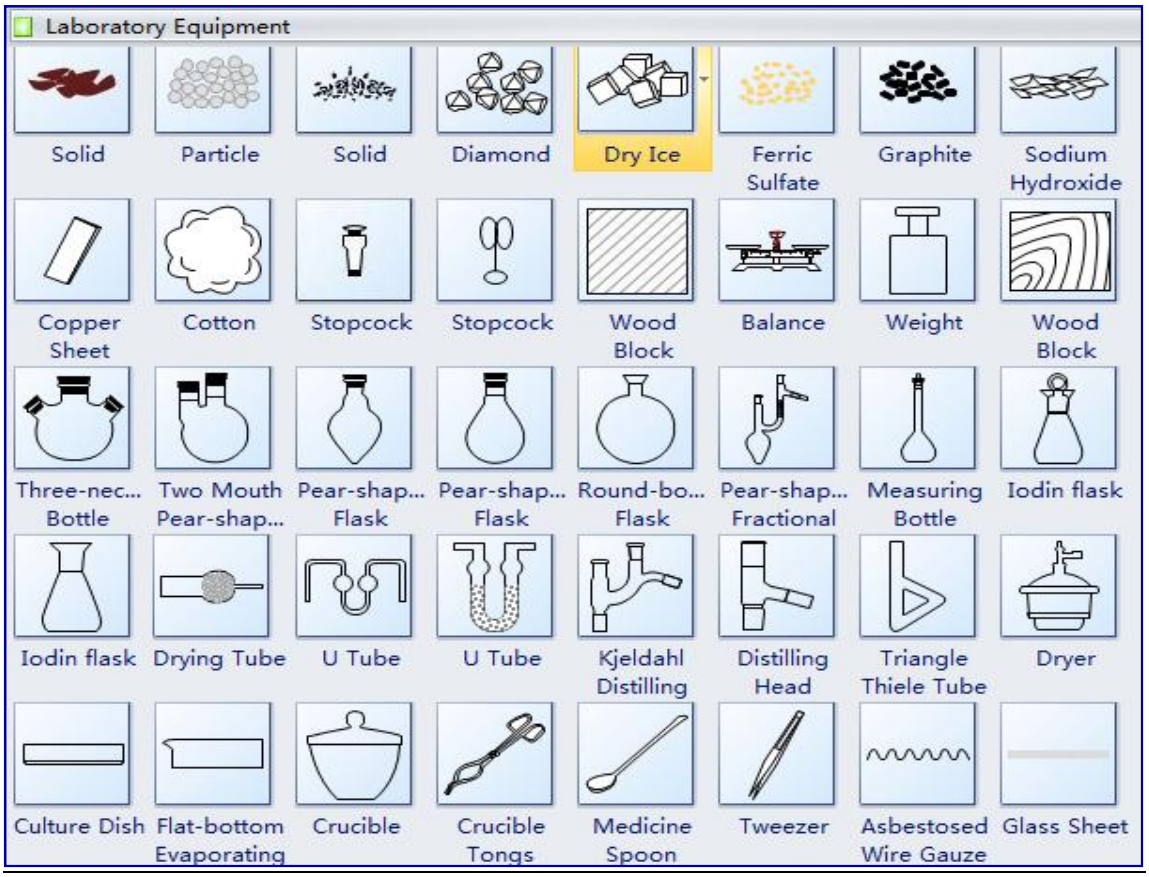
4. COMMON LABORATORY EQUIPMENTS, APPARATUSES AND GLASS WARES



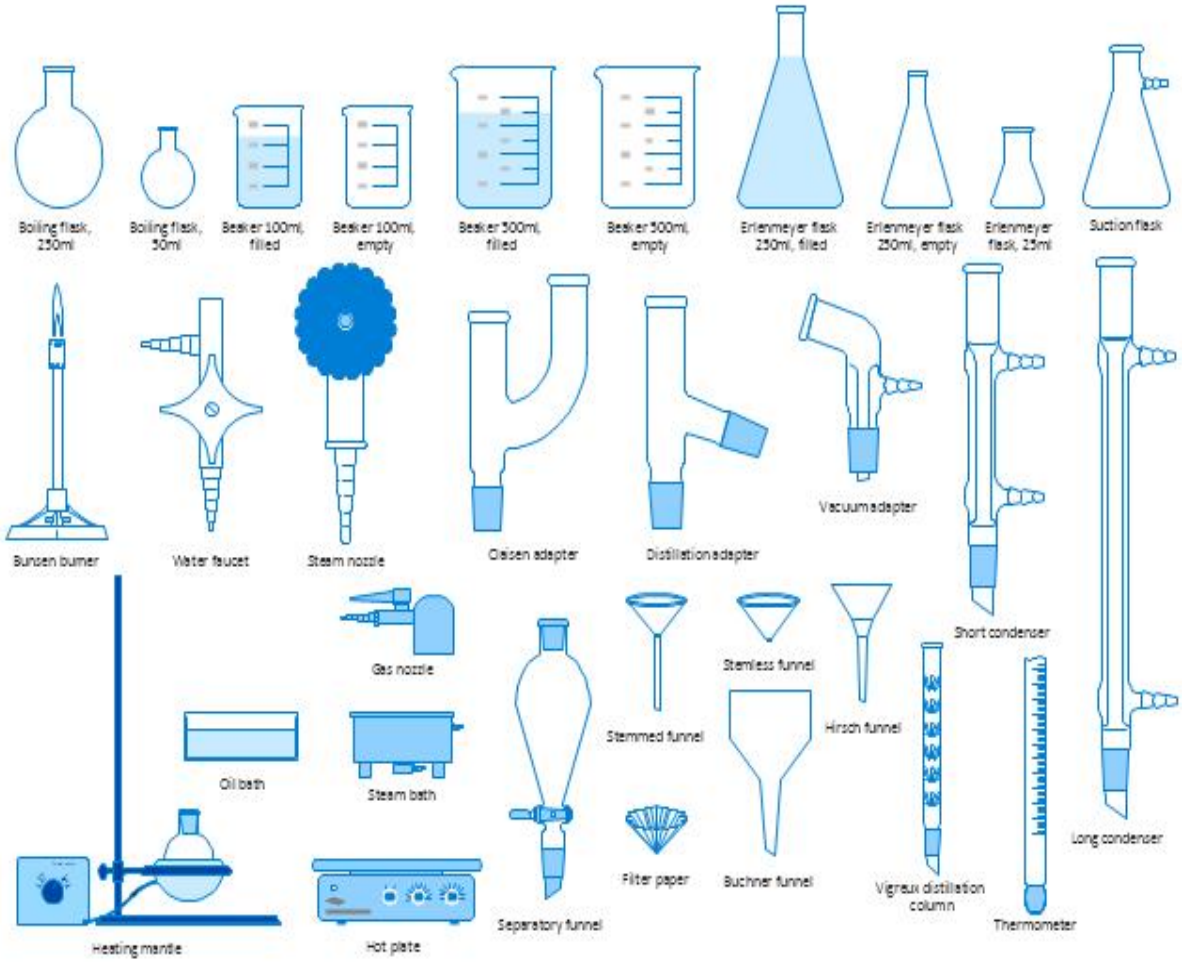

Beaker
Mortar and pestle
Crucible and cover
Watch glass
Evaporating dish
Pneumatic trough
Florence flask
Wide-mouth collecting bottle
Plastic wash bottle
Dropper pipet
Funnel
Erlenmeyer flask
Safety goggles
Rubber stoppers
Glass rod with nichrome wire (for flame testing)
Test-tube holder
Clay triangle
Ring stand
Scoopula
Crucible tongs
Rubber tubing
Metal spatula
Ceramic square
Wire gauze
Test-tube brush
Triangular file
Burner
Tripod

LABORATORY APPARATUSES





SOME LABORATORY GLASS WARES



5. LASSAIGNE`S EXTRACT (SODIUM FUSION EXTRACT) AND DETECTION OF ELEMENTS

Lassaigne`s extract is prepared for the detection of nitrogen, sulphur, and halogens (Cl, Br, I) in an organic compound. These elements are covalently bonded to the organic compounds. In order to detect them, these have to be converted into their ionic forms. This is done by fusing the organic compound with sodium metal (Na). The ionic compounds formed during the fusion are extracted in aqueous solution and can be detected by simple chemical tests. The extract is called sodium extract, sodium fusion extract or Lassaigne's extract.

Organic Compound (containing C, H, O, N, S, X) + Na $\longrightarrow$ $NaCN + Na_2S + NaX + NaOH$
(Where, X = Cl, Br or I)

Test for nitrogen: The carbon and nitrogen present in the organic compound on fusion with sodium metal give sodium cyanide (NaCN) soluble in water. This is converted in to sodium ferrocyanide by the addition of sufficient quantity of ferrous sulphate. Ferric ions generated during the process react with ferrocyanide to form blue precipitate of ferric ferrocyanide.

$$Na + C + N \longrightarrow NaCN$$

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

Sodium ferrocyanide

$$Na_4[Fe(CN)_6] + Fe^{3+} \longrightarrow Fe_4[Fe(CN)_6]_3$$

Ferric ferrocyanide

Test for sulphur: If sulphur is present in the organic compound, sodium fusion will convert it into sodium sulphide. Sulphide ions are readily identified using sodium nitroprusside appearance of a deep violet colour indicates sulphur.

$$Na + S \longrightarrow Na_2S$$

$$Na_2S + Na_2[Fe(CN)_5NO] \longrightarrow Na_4[Fe(CN)_5NOS]$$

Sod. nitroprusside — Sod. thio-nitroprusside (purple-violet colour)

The presence of sulphur can also be identified by appearance of black precipitate (lead sulphide) after the addition of lead acetate.

$$(CH_3COO)_2Pb + Na_2S \longrightarrow 2\,CH_3COONa + PbS$$

Test for both Nitrogen & Suphur together:

If both N & S are present in the sample, then sodium thiocyanate (NaSCN) is formed with sodium (ionic form of nitrogen and sulphur together in the extract). Sodium thiocyanate

(NaSCN) reacts with ferric chloride to give ferric thiocyanate, a blood red colour complex. Thus, the appearance of blood red colour indicates the presence of nitrogen and sulphur together.

$$3NaSCN + FeCl_3 \longrightarrow Fe(SCN)_3 + 3NaCl$$

Test for halogens (Cl, Br, I): Halogens (X) react with Na to form sodium halide (NaX). The sodium halide (NaX) is reacted with silver nitrate ($AgNO_3$) to give precipitate of silver halide (AgX). A white precipitate (AgCl) soluble in ammonium hydroxide indicates the presence of chlorine. An off-white white precipitate (AgBr) partly soluble in ammonium hydroxide indicates the presence of bromine, while yellow precipitate (AgI) insoluble in ammonium hydroxide indicates the presence of iodine.

$$NaX + AgNO_3 \longrightarrow AgX\downarrow + NaNO_3$$

PROCEDURE FOR LASSAIGNE'S EXTRACT (LE):

Take a small piece of sodium metal with spatula and dry it in between the folds of filter paper. Place the piece of sodium metal in an ignition tube and melt it, then add small amount of the given sample in the ignition tube. Heat the ignition tube over the flame till it become re hot. Take 20 mL of distil water in a china dish and crush the red-hot ignition tube (4-5 tubes) in it. Boil the mixture for 5 minutes and then filter it. This colourless filtrate is called sodium extract/sodium fusion extract/Lassaigne`s extract (LE). Perform different tests taking 1-2 mL of the extract for detecting the presence of element(s). A coloured filtrate indicates incomplete decomposition and the entire fusion procedure should be repeated.

Chemical tests for detection of element(s):

Test for Nitrogen	**Observation**
Take 1-2 mL Lassaigne`s extract (LE) in a test tube, add 2-3 crystals of ferrous sulphate ($FeSO_4$) and boil it. Add few drops of NaOH. A dirty green ppt is formed. Now add 2-3 drops of conc. H_2SO_4 by the walls of the test tube. The appearance of a Prussian blue colour (ferri-ferrocyanide) indicates the presence of nitrogen.	Prussian blue colour
Test for Sulphur	
1. Take 1-2 mL LE and add few drops of sodium nitroprusside, if sulphur is present then sodium thio-nitroprusside is formed which is purple-violet in colour. 2. Take 1-2 mL LE and add lead acetate containing acetic acid. The formation of a black precipitate (PbS; lead sulphide) indicates the presence of sulfur.	Purple-violet colour Black precipitate

Nitrogen and Sulphur present together	
Take 1-2 mL LE and add 2-3 crystals of ferric chloride. If both nitrogen and sulphur is present then ferric thiocyanate is formed (blood red colour).	Blood red colour
Test for halogens (Cl, Br, I)	
To 1-2 mL LE, add 1-2 drops of HNO_3 and then add $AgNO_3$ solution. (i) The formation of a white precipitate (AgCl) that is <u>soluble</u> in NH_4OH indicates the presence of chlorine in the given sample. (ii) The formation of an off-white precipitate that is <u>partially soluble</u> in NH_4OH confirms the presence of bromine. (iii) Where as the formation of a yellow precipitate <u>insoluble</u> in NH_4OH confirms the presence of iodine in the given sample.	White precipitate Off-white precipitate Yellow precipitate

Precautions:

1. Sodium metal is highly reactive, when exposed to air, it reacts even with the moisture present in the atmosphere. It also reacts with the sweat of hands. Therefore, DO NOT hold it with hands. Always use dry forceps while handling sodium metal.
2. Before using sodium metal in any experiment, press it within the folds of filter paper to remove oil.
3. Use dry ignition tubes for sodium fusion. Sodium reacts with water violently.
4. Put the unused sodium metal piece back in its container. DO NOT throw it into the sink or dustbin.
5. Repeat the process of sodium fusion with at least three ignition tubes. This is to ensure that the fusion has taken place.
6. After immersing the red hot ignition tube in water, break it with glass rod gently. Boil the contents for 2-3 minutes so as to extract the soluble sodium salts in water.

6. GENERAL PATTERN OF FUNCTIONAL GROUP TESTS

The identification of organic substances is a major subject in organic chemistry. It involves physical (melting point, boiling point, solubility, etc.) as well as chemical investigation.The quantitative analysis of organic compound is much more difficult & complicated than that of inorganic compounds. The fundamental groups determinations are depend on the correct determination of elements. Once the functional group present in the organic compound is known one is able to find out the name of compound with the help of melting point & boiling point.

Inorganic compound are generally soluble in water. In aqueous solution majority of each compound dissociate giving ion which can be easily recognize by applying simple test. Once these ions are known, their inorganic compound under examination can be identified. On the other hand majority of organic compound do not dissolve in water, further organic compound being covalent do not dissociate in solution giving ions this makes the recognition of organic compound more difficult.

The number of organic compound contain the same functional group such as –COOH, - OH, - NO_2 etc. all such compound are generally solid to belong the particular class of organic compounds. These have similar chemical properties e.g:- acetic acid, propionic acid & butyric acid belong to the same class because they contain the same functional group namely carboxylic group. All the three acid therefore have almost similar properties & so it is very difficult to identify them by applying chemical test. In such case physical properties like melting point, boiling point etc. are used to recognize the given organic compound. Since organic compound are very large in number their analysis can be tedious & time consuming if we do not proceed systematically step by step, its detection is simply, we should proceed systematically to recognize organic compound.

The unknown sample is generally analyzed by a series of tests like- preliminary tests, solubility in different solvents, ignition test, test for unsaturation, element detection and finally specific functional group tests. Let’s see how these tests are performed and what information we get from these tests.

A. Preliminary tests (Physical characteristics):

Nature: Solid/liquid/semisolid

Colour: colourless/transparent or specific colour

Odor: odorless/pungent/fruity/sweet/bad

Solubility: soluble or insoluble in different solvents like- water, HCl, NaOH, $NaHCO_3$.

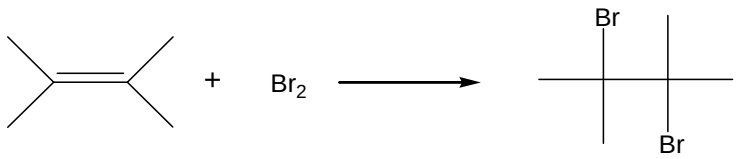
The solubility of a compound in different reagents (solvents) gives important information about its category/class. Take 0.1 g or 2-3 drops of the given sample in about 3 mL of a solvent in a test tube and shake well, observe its solubility (see the following table).

Solvent/reagent & test	**Nature of compound**	**Class of compounds**
Soluble in water (cold or hot). Further solubility test is not done if the sample is soluble in water.	Neutral, acidic or basic (also test with litmus or universal indicator paper)	Lower members of series. Neutral- alcohols, Acidic- acids, phenols, Basic- amines.
Soluble in HCl	Basic	Amines
Soluble in NaOH	Acidic	Acids, phenols
Soluble in $NaHCO_3$ (sod. bicarbonate)	Strongly acidic	Carboxylic acids
Insoluble in above solvents	Neutral	Hydrocarbons, alkyl/aryl halides, esters & ethers, higher alcohols, aldehydes & ketones

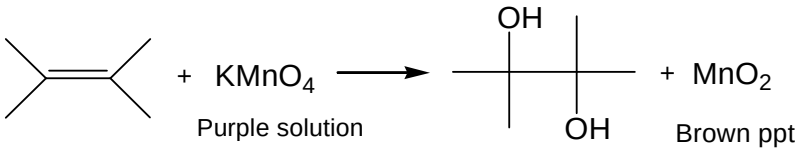
A. ***Ignition test*****:** Burn a small amount of the sample on a metal spatula to know about the aliphatic or aromatic nature of the compound. Luminous flame indicates aliphatic compound while sooty/smoky flame indicates aromatic compound.

C. *Test for unsaturation*: These tests are done to know whether the compound is saturated or unsaturated.

(i) **Bromine test**: Take 0.1 gm or 2-3 drops of the given sample in a test tube and add 2 mL of chloroform/carbon tetra chloride/methylene chloride. Add a solution of bromine in chloroform/carbon tetra chloride/methylene chloride dropwise with shaking. Disappearance of bromine colour indicates unsaturation in the sample. The bromine gets attached across the double and triple bonds (unsaturation) and makes them single bonds (saturated).



(ii) **Potassium permanganate test (Baeyer test)**: Take 0.1 gm or 2-3 drops of the given sample in a test tube and add 2 mL of acetone. Add 1% aqueous solution of $KMnO_4$ dropwise with shaking. Disappearance of purple colour of $KMnO_4$ indicates unsaturation or oxidizable functional group. Appearance of little quantity of brown ppt is MnO_2.



D. ***Detection of elements present:***

Prepare Lassaigne's extract (sodium extract) and test for the presence of elements (nitrogen, sulphur, halogens).

E. ***Functional group determination:***

The above tests often indicate the class/functional groups present in the given sample. Specific tests are then performed to identify and confirm the functional group present. In the following pages specific tests for functional groups like carboxylic (-COOH), alcohol (R-OH), phenol (Ar-OH), amino ($-NH_2$), etc. are given.

7. FUNCTIONAL GROUP TEST (TEST FOR CARBOXYLIC ACID)

The functional group of a carboxylic acid is a carboxyl group. The general formula for an aliphatic carboxylic acid is RCOOH and for an aromatic carboxylic acid is ArCOOH. Carboxylic acids have significantly higher boiling points than other types of organic compounds of comparable molecular weight. They are polar compounds and form very strong intermolecular hydrogen bonds. Carboxylic acids are more soluble in water than alcohols, ethers, aldehydes, and ketones of comparable molecular weight. They form hydrogen bonds with water molecules through both their C=O and OH groups. They are dissolving in Na_2CO_3 with evaluation of CO_2; they are also dissolving in NaOH. Carboxylic acids are divided into two categories, aliphatic and aromatic carboxylic acids.

S. No.	Test	Observation
i.	**Litmus paper test** Take sample and dissolve in water/alcohol. Dip a blue litmus paper and see the colour change.	Blue litmus turns red
ii.	**Sodium bicarbonate test (Effervescence test)** Take sample and dissolve in water, add a pinch of sodium bicarbonate and observe for effervescence. $RCOOH + NaHCO_3 \longrightarrow RCOONa + CO_2\uparrow$	Effervescence of CO_2 is observed
iii.	**Neutral ferric chloride test** Make ferric chloride solution neutral by addition of NaOH and add this solution to sample solution. Observe for change in colour.	Red colour complex is formed
iv.	**Ester formation test** A mixture of 0.2 gm of the sample, 0.4 mL of ethanol, and 0.2 mL of conc. H_2SO_4 is heated on water bath for 2 min. Pour the mixture slowly into a evaporating dish containing 2 mL of sodium bicarbonate solution. $RCOOH + R`OH \longrightarrow RCOOR` + H_2O$	Fruity/sweet smell indicates that the carboxylic group has been esterified.

Formic acid

HCOOH

Physical properties:

Molecular formula CH_2O_2, colorless liquid, miscible with water, alcohol and ether, b.p. 100 °C.

Chemical properties

1- It reduces Fehling`s solution and Tollen`s reagent.

2- It decolorizes $KMnO_4$.

3- With $FeCl_3$: Aq. solution of acid gives red color which is converted to brown by boiling.

4- Ester formation: to 1 mL of acid add 1 mL of ethyl alcohol and 1 mL of conc. H_2SO_4 in attest tube, heat in water bath, and then pour to Na_2CO_3 solution .the characteristic odor of ethyl format is evolved.

Acetic acid

CH_3COOH

Physical properties:

Molecular formula $C_2H_4O_2$, colorless viscous liquid, miscible with water, alcohol and ether, b.p 122 °C.

Chemical properties:

1- It does not reduce Fehling`s solution and Tollen`s reagent.

2- With $FeCl_3$: Aq. solution of acid gives red color which is converted to brown by boiling.

3- Ester formation: to 1 mL of acid add 1 mL of ethyl alcohol and 1 mL of conc. H_2SO_4 in a test tube, heat on water bath, and then pour to Na_2CO_3 solution. The characteristic odor of ethyl acetate is evolved.

Oxalic acid

Physical properties:

Molecular formula $C_2H_2O_4$, colorless crystalline solid, m.p. 100 °C, soluble in water and alcohol.

Chemical properties

1- Flaming test. When the acid or its salt is heated on a piece of porcelain it is decomposed with little or no charring.

2- When the acid is heated with conc. H_2SO_4 it is decomposed into CO and CO_2 with no charring.

3- Aq. solution + $CaCl_2$: A white ppt. of Ca oxalate is separated immediately on cold which is soluble in mineral acids.

4- Aq. solution + $AgNO_3$: gives white ppt. of Ag oxalate.

5- When heated with few drops of dil. $KMnO_4$ solution and then acidified- the color is discharged.

Tartaric acid

Physical properties:

Molecular formula $C_4H_6O_6$, colorless crystalline solid, m.p. 167 °C, soluble in cold water and alcohol.

Chemical properties

1- It gives positive acidity test.

2- With conc. H_2SO_4: when the solid is heated with conc. H_2SO_4 charring is occur with the evaluation of odor of burnet sugar.

3- Aq. solution + $CaCl_2$: it gives white ppt. after shaking from calcium tartrate, which is soluble in mineral acids.

4- Aq. solution + $AgNO_3$: it gives Ag mirror after heating in water bath.

5- $KMnO_4$ + aq. solution of tartaric acid, heat in presence of dil. H_2SO_4, decolorization of color occurs.

Citric acid

Physical properties:

Molecular formula $C_6H_8O_7$, colorless crystalline solid, m.p 100 °C, soluble in cold water

Chemical properties:

1- With conc. H_2SO_4: heating the solid with conc. H_2SO_4 gives yellow color.

2- It gives Acidity test +ve.

3- with $CaCl_2$ solution: it gives white ppt. after boiling.

4- Deng`s test: 1 mL of $HgSO_4$ solution is added to 5 mL of neutral solution. Then heat to boiling and then add 1-2 drops of 2% $KMnO_4$ where decolorization occurs and a heavy white ppt appears.

Benzoic acid

Physical properties:

Molecular formula $C_7H_6O_2$, white crystalline solid, mp. 121°C, insoluble in cold water but soluble by boiling and re precipitated by cooling, soluble readily in alcohol.

Chemical properties

1- It gives Acidity test +ve.

2- Aq. solution + $FeCl_3$: gives buff ppt.

3- It gives ester test with ethyl alcohol.

4- It gives soda lime test: In dry test tube, place a layer of soda lime powder, then layer of benzoic acid and then another layer of soda lime. Then heat and note the odor of benzene.

Salicylic acid

Physical properties:

Molecular formula $C_7H_6O_3$, colorless solid, m.p. 159°C, soluble in alcohol, ether, to some extent it is soluble in water.

Chemical properties

1- It gives Acidity test +ve.

2- Aq. solution + $FeCl_3$: gives violet color.

3- It gives ester test with methyl alcohol.

4- It gives soda lime test: In dry test tube, place a layer of soda lime powder, then layer of benzoic acid and then another layer of soda lime. Then heat and note the odor of phenol.

Cinnamic acid

Physical properties:

Molecular formula $C_9H_8O_2$, white crystals, mp. 133°C, sparingly soluble in water but soluble in alcohol.

Chemical properties

1- It gives Acidity test +ve.

2- Aq. solution + $FeCl_3$: gives buff ppt.

3- It gives ester test with ethyl alcohol.

4- Unsaturation test: Dissolve 2 g of acid in Na_2CO_3 solution (5 mL) adds 1% aqueous $KMnO_4$ solution drop wise immediate decolonization is observed.

5- Dissolve 2 gm of acid or its salt in Na_2CO_3 solution (5 mL) add Br water drop wise and note the separation of bromostyrene as color oil.

Phthalic acid

Physical properties:

Molecular formula $C_8H_7O_4$, white solid, m.p. 191°C, it forms phthalic anhydride which dissolves in water, readily soluble in hot water and organic solvents.

Chemical properties

1- It gives Acidity test +ve.

2- Aq. solution + $FeCl_3$: gives buff ppt.

3- It gives fluorescein reactions with resorcinol where it gives red solution with instance green fluorescein.

4- It gives phthaline reaction with phenol where it gives bright red color.

8. FUNCTIONAL GROUP TEST (TEST FOR ALCOHOL)

Alcohols are classified as primary, secondary and tertiary according to the number of alkyl groups directly attached to the carbinol carbon. The general formula of an alcohol is R-OH in which the R is an aliphatic hydrocarbon group. Alcohols may be looked upon as derivatives of water, HOH. One hydrogen atom of water is substituted by an alkyl group (R). Like water, alcohols show hydrogen bonding. As the chain of the R group increases the hydrocarbon character of the compound overshadows the polar character of the OH group. Consequently, the solubility and boiling point of an alcohol are affected by the length of the carbon chain and the shape of the molecule.

The shorter chain alcohols are water soluble, while the long chain alcohols are not soluble in water. Phenols are aromatic alcohol where the R group is aromatic ring. Reactions of alcohols involve the breaking of either of two bonds: the O-H bond as in reactions with bases and esterification reactions, or the C-OH bond leading to dehydration and substitution reactions. In breaking the COH bond, protonation of the -OH group is essential to convert it from a poor leaving group to a better one.

Ethanol, methanol, isopropanol are examples of monohydric alcohols. Ethylene glycol is an example of dihydric alcohols. Glycerol is an example of trihydric alcohol.

S. No.	Test	Observation
i.	***Sodium detection of active hydrogen*** $2ROH + 2Na \longrightarrow 2RO^-Na^+ + H_2$ (gas)	
	To 0.25 mL or 0.25 gm of the sample, add small thin slices of freshly cut sodium until no more is dissolved (saturated). Evolution of hydrogen gas indicates the presence of an acidic hydrogen, such as a hydroxyl group in an alcohol, hydrogen attached to the nitrogen in a primary or secondary amine, or hydrogen in a terminal alkyne.	Hydrogen gas evolved
ii.	**Cerric ammonium nitrate** $ROH + (NH_4)_2Ce(NO_3)_6 \longrightarrow (NH_4)_2CeOR(NO_3)_5 + HNO_3$	
	To 1 mL of the cerric ammonium reagent, add 4-5 drops of the given sample (if liquid) or 0.1-0.2 gm (if solid). Mix thoroughly and note the colour change.	yellow colour of the reagent changes to red

iii.	**Chromic anhydride (Jones oxidation)** $3R_2CHOH + 2CrO_3 + 3H_2SO_4 \longrightarrow RCOR + 6H_2O + Cr_2(SO_4)_3$	
	To 1 mL of acetone in a small test tube, add 1 drop of the liquid or about 10 mg of a solid compound. Then add 1 drop of the Jones reagent and note the result within 2 sec.	Precipitate
iv.	**Lucas test** $(R)_3\text{-}OH + HCl \longrightarrow (R)_3\text{-}Cl + H_2O$	
	To 0.25 mL or 0.25 gm of the sample in a test tube, add 2 mL of Lucas reagent (13.6 gm of anhyd. Zinc chloride in 10.5 g of conc. HCl). Stopper the tube and shake, then allow to stand. Note the time required for the formation of the alkyl chloride, which appear as an insoluble layer or emulsion.	Appearance of a cloudy second layer or emulsion (time taken). 3º alcohols- immediate to 3 min. 2 º alcohols- 5 to 10 min. 1 º alcohols- no rxn.
(v)	**Acetyl chloride test** $Ar\text{-}OH + CH_3COCl \longrightarrow Ar\text{-}O\text{-}CO\text{-}CH_3 + HCl$ (gas)	
	Add drop by drop 0.2 mL of acetyl chloride to 0.2 mL or 0.2 gm of the sample. Allow the mixture to stand for 1-2 minute and then pour it cautiously into 1 mL of water.	Evolution of heat and HCl gas or a ppt. Alcohols produce esters indicated by fruity/sweet smell.

Methyl alcohol

CH_3OH

Physical properties:

Molecular formula CH_4O, colorless liquid, b.p 65 °C, miscible with water.

Chemical properties

1- Ester formation

1- In dry test tube put 1 mL of methyl alcohol then add 0.5 mL of conc. H_2SO_4 and 0.5 gm of salicylic acid or its derivatives.

2- Heat the mixture for 3 min on water bath, then cool and pour the contents in a beaker containing about 30 mL of sodium carbonate solution.

3- Note the characteristic odor of methyl salicylate.

2- Oxidation reaction

1- In dry test tube, place 0.5 mL of $K_2Cr_2O_7$ and 0.5 mL of conc. H_2SO_4, then cool.

2- Add 0.5 mL of methanol and boil gently (on water bath) notice the pungent odor of formaldehyde and change of color to green.

Ethyl alcohol

CH_3CH_2OH

Physical properties:

Molecular formula C_2H_6O, colorless liquid, miscible with water, b.p 78 °C.

Chemical properties

1- Oxidation reaction

1- Place 1 mL of $K_2Cr_2O_7$ and 0.5 mL of conc. H_2SO_4, then cool.

2- Add 0.5 mL of ethanol and boil gently (on water bath)

3- Notes the odor of acetaldehyde and change the color solution to green.

2- Ester formation

1- Place 1 mL of ethanol in dry test tube, then add 0.5 mL of conc. H_2SO_4 and 1 mL of acetic acid.

2- Heat the mixture gently for 3 mints in water bath, cool and pour the tube into baker containing sodium carbonate solution.

3- Not the characteristic odor of ester.

3- Iodoform test

1- Add 3 mL of iodine solution to 1 mL of ethyl alcohol then add NaOH solution drop wise until the color of solution becomes straw yellow.

2- Heat the solution in water bath for 5 mints.

3- Leaves it to cool gradually, a yellow ppt. of iodoform is appearing

Glycerol

Physical properties

Colorless viscous liquid, odorless, has sweet taste, miscible with water and alcohol in all proportions.

Chemical properties

1- Oxidation reaction

Glycerol oxidized to give several products but it is ultimately transformed into CO_2 and H_2O.

1- Add 2 mL of conc. H_2SO_4 and 2 mL of $K_2Cr_2O_7$, then cool.

2- Add 0.5 mL of glycerol and boil gently (on water bath).

3- Notice the effervescence due to the evolution of CO_2.

2- Acroline test

1- Heat 0.5 gm of glycrine with 1gm of hydrogen potassium sulphate $KHSO_4$ or 2 mL of conc. sulphuric acid in dry test tube.

2- Notice odor of acroline.

3- Borax test

1- Add one drop of ph.ph to 1 mL of dil. Borax solution red color appears.

2- Add 1 mL of glycerol and note that the color disappears,

3- Heat gently and observe the appearance of the red color once more, which disappear on cooling the solution.

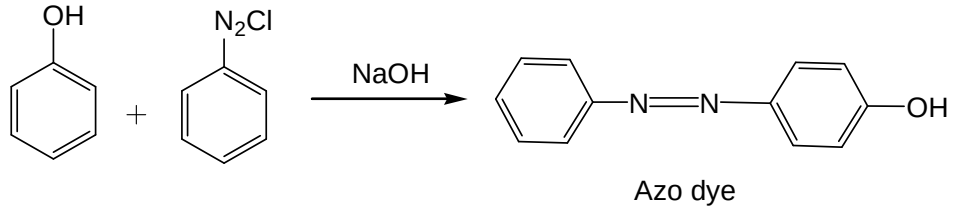
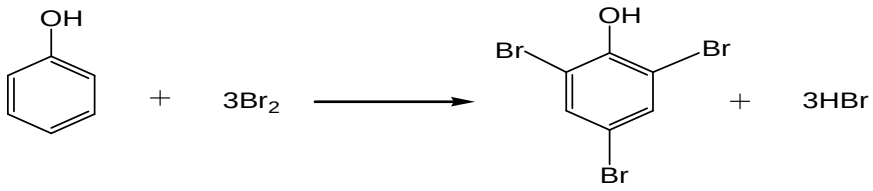
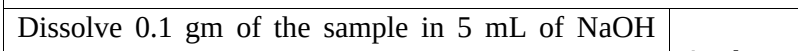
9. FUNCTIONAL GROUP TEST (TEST FOR PHENOLS)

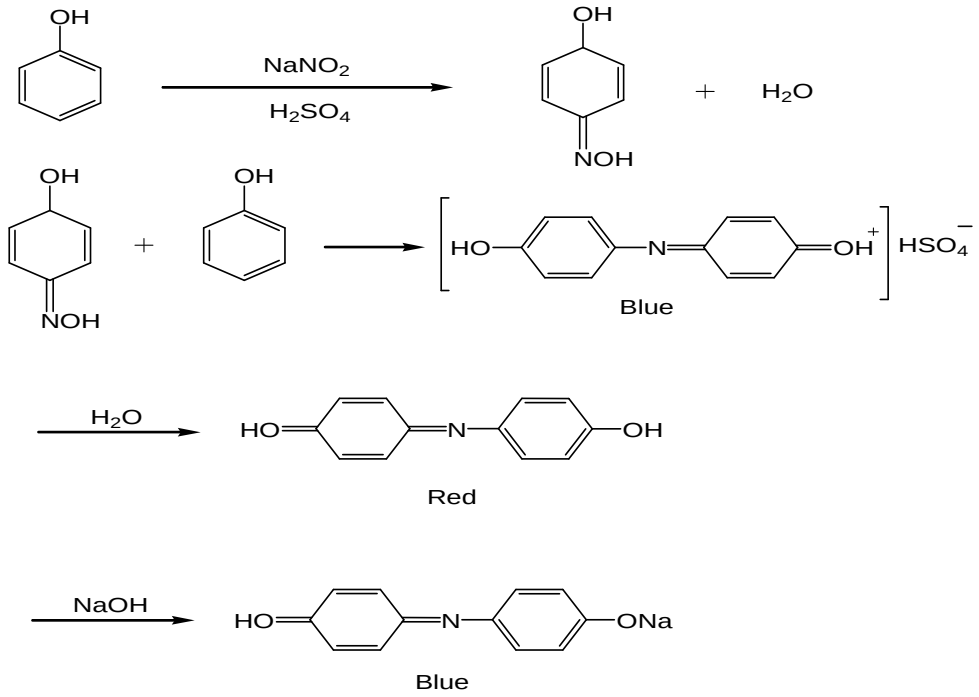
Phenols are hydroxyl aromatic compounds which dissolve in alkali forming phenolates. Phenols are classified into mono-, di-, and tri hydric according to the number of OH groups; Monohydric: such as phenol, β- and α- naphthol. Dihydric: such as catechol, resorcinol and hydroquinol. Trihydric: such as pyrogallol.

The most common reactions of phenols involve breaking the O-H bond and the usual electrophilic aromatic substitution at the aromatic ring. Protonation of the hydroxyl group and loss of a water molecule as in alcohols would give a phenyl cation which is very unstable and difficult to form. Since the aromatic nucleus is electron rich, direct attack by nucleophiles as in SN_1or SN_2 reactions is not possible. Consequently, phenols do not undergo substitution of the hydroxyl group either by the SN_1 or SN_2 mechanisms.

The characteristic property that differentiates phenols from alcohols is acidity. Phenols are stronger acids than alcohols and react with sodium hydroxide, whereas alcohols do not. The reason for this difference is that the phenoxide ion is resonance-stabilized whereas the alkoxide ion is not.

S. No.	Test	Observation
i.	***Ferric Chloride Test*** $3ArOH + FeCl_3 + 3$ pyridine $\longrightarrow$ $Fe(OAr)_3 +$ 3 pyridinium hydrochloride Coloured complex	
	Dissolve 0.05 gm of the sample in 2 mL of water (if the sample does not dissolve, even partially, dissolve it in a mixture of water and ethanol/pyridine) and an aqueous solution neutral ferric chloride drop wise. Shake the test tube and note the colour produced immediately.	Phenols form characteristic coloured iron complexes when treated with neutral ferric chloride. E.g. phenol & resorcinol – violet colour, catechol – green.
ii.	**Azo dye test**	

	 Azo dye	
	Dissolve two drops of aniline in 1 mL dil. HCl, cool in ice and add saturated solution of sodium nitrite. Now it is added to a well cooled solution of the sample (phenols) in aqueous sodium hydroxide.	A red coloured dye is formed. Aryldiazonium salts react with aromatic rings of phenols to form highly coloured azo compounds (dye). These rxns are called coupling rxns.
iii.	**Bromine Water** 	
	Dissolve 0.1 gm of the sample in 10 mL of water. Add bromine water drop by drop until the bromine colour is no longer discharges. 2,4,6-Tri-bromo-phenol is formed as white ppt.	Discharging of the bromine colour or formation of a white precipitate is a positive test.
iv.	**Benzoylation test** 	
	Dissolve 0.1 gm of the sample in 5 mL of NaOH (20% w/v). Add 1 mL of benzoyl chloride and shake vigorously with caution for about 15 min.	A white ppt (ester) is formed.

v.	**Liebermann's Nitroso reaction**  	
	Add a crystal of sodium nitrite to 2 mL of conc. sulfuric acid, and shake until it dissolves. Add 0.1 gm of the given sample. A blue colour indicates the presence of a phenol. Pour into ice water, red colour reappears, make alkaline by adding 10% sodium hydroxide solution blue colour reappears.	Blue colour

Phenol

Physical properties:

Molecular formula C_6H_6O, colorless crystalline (when pure), m p. 43°C, it is poisonous and corrosive, it turns red in air.

Chemical properties

1- With ferric chloride it gives violet color discharged with HCl

2- With phthaline test gives a deep pink color.

3- With azo - dye it gives red ppt.

4- With bromine water a white ppt. is formed.

5- With excess of bromine yellowish white of tri – bromo phenol is formed.

Hydroquinol

Physical properties:

Molecular formula $C_6H_6O_2$, soluble in water, m.p. 170 °C, it is sparingly soluble in benzene.

Chemical properties

1- With ferric chloride it gives green needles.

2- With bromine water it gives yellowish white ppt.

3- Does not give azo dye test.

4- With phthaline reaction it gives blue violet color.

5- It reduces Fehling`s solution and Tollen`s reagent.

Catechol

Physical properties:

Molecular formula $C_6H_6O_2$, colorless crystals, soluble in water, alcohol, m.p. 105 °C.

Chemical properties

1- With ferric chloride it gives green color.

2- With bromine water it gives deep red color.

3- Does not give azo dye test

4- With phthaline reaction it gives blue color.

5- It is reduces Fehling`s solution and Tollen`s reagent.

Resorcinol

Physical properties:

Molecular formula $C_6H_6O_2$, coluble in water, m.p. 110 °C.

Chemical properties

1- With ferric chloride it gives deep violet color.

2- With bromine water it gives white ppt. dissolved in excess.

3- Gives with azo dye test red ppt.

4- With phthaline reaction it gives reddish fluorescence solution.

5- It reduces Fehling`s solution and Tollen`s reagent.

6- With conc. Nitric acid it gives red color.

Pyrogallol

Physical properties:

Molecular formula $C_6H_6O_3$, it is plates or needles, soluble in water and alcohol, m.p. 132 °C.

Chemical properties

1- With ferric chloride it gives reddish color and in very dil. solution of NaOH it gives violet color.

2- Solution of pyrogallol + glycerol (solution) + conc. H_2SO_4 it gives reddish violet color.

3- Solution of pyrogallol + HCHO + conc. HCl gives white ppt. turns to pink.

4- It reduces Fehling s solution and Tollen`s reagent.

α- Naphthol

Physical properties:

Molecular formula $C_{10}H_8O$, soluble in alcohol, ether and benzene, mp. 94 °C.

Chemical properties

1- With ferric chloride it gives greenish color at first rapidly turns violet on adding excess.

2- It decolorizes bromine water and there occurs no ppt.

3- Gives with azo dye test brownish red ppt.

4- With phthaline reaction it gives green solution.

β- Naphthol

Physical properties:

Molecular formula $C_{10}H_8O$, soluble in alcohol, ether and benzene, m.p. 123°C.

Chemical properties

1- With ferric chloride it gives greenish color at first rapidly turns violet on adding excess.

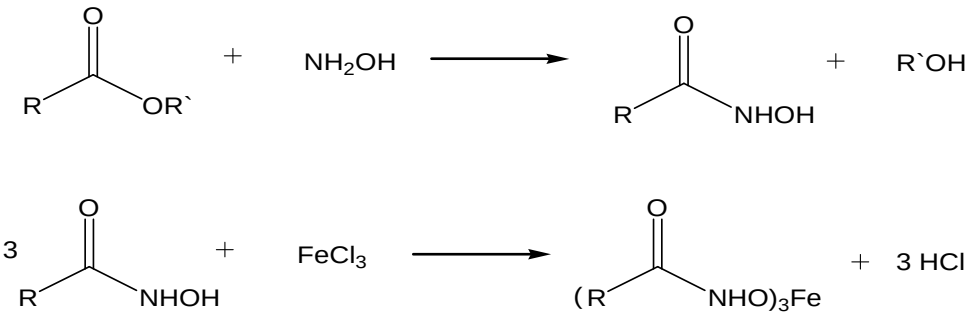
2- With bromine water no ppt. is formed but the color disappears.

3- Gives with azo dye test scarlet red ppt.

4- With phthaline reaction it gives faint blue with slight fluorescence.

10. FUNCTIONAL GROUP TEST (TEST FOR ESTERS)

Esters are chemical compounds consisting of a carbonyl adjacent to an ether linkage. They are derived by reaction of an acid with a hydroxyl compound such as an alcohol or phenol. Esters are usually derived from an inorganic acid or organic acid in which at least one -OH (hydroxyl) group is replaced by an –O-alkyl (alkoxy) group, and most commonly from carboxylic acids and alcohols. That is, esters are formed by condensing an acid with an alcohol.

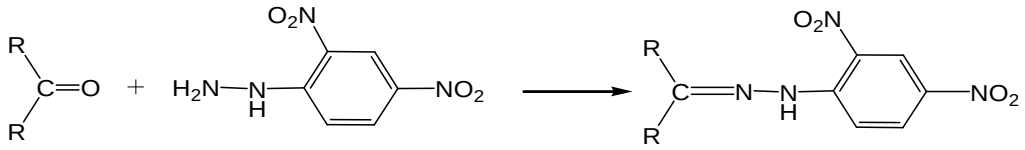
S. No.	Test	Observation
i.	**Hydroxamic acid test**  	
	Mix 1 drop of the compound in 1 mL of methanolic solution of hydroxylamine hydrochloride, add few drops of methanolic KOH to make it alkaline, heat to boil. Cool to room temperature and cautiously add 2 mL of 1M hydrochloric acid. Add one drop of 5% ferric chloride solution and observe colour.	Deep red or violet colour of the ferric hydroxamate is produced
ii.	**Hydrolysis test (Phenolphathalein test)** R–COO–R` ⟶ R–COONa + R`–OH	
	Dissolve a little quantity of sample in 2 mL of methanol and add few drops of dil. NaOH and one drop of phenolphthalein. A pink colour is produced, heat the test tube gently on water bath and observe the colour. The colour starts to fade and finally disappears.	A pink colour is produced which fades upon heating

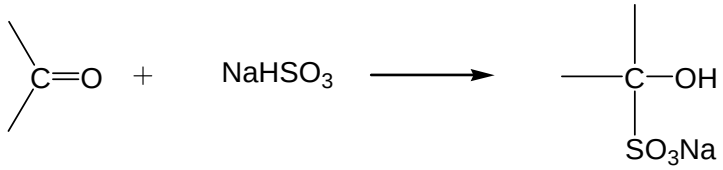
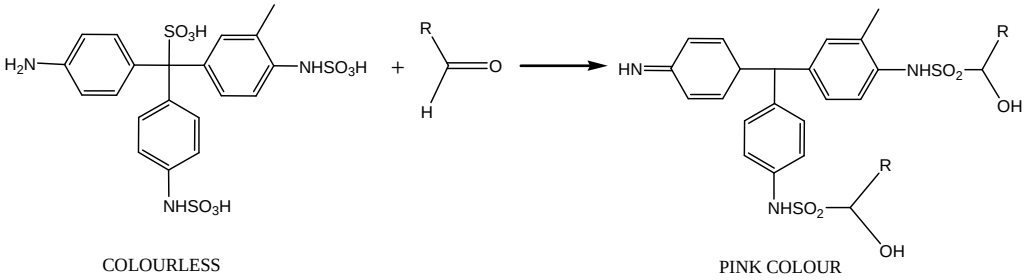
11. FUNCTIONAL GROUP TEST (TEST FOR CARBONYL GROUP: ALDEHYDE AND KETONE)

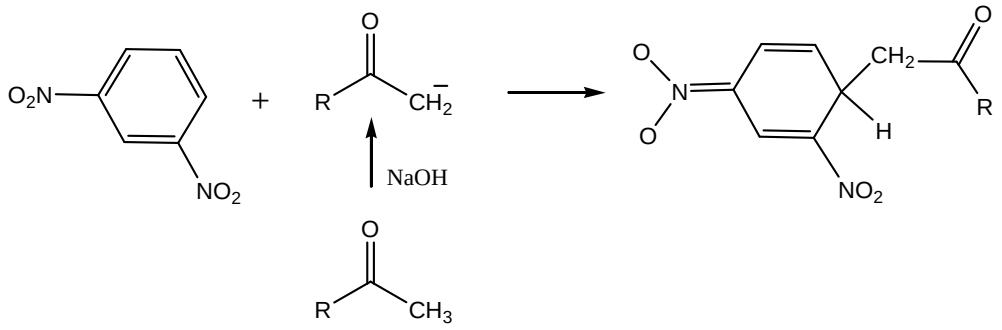
The carbonyl group is common to both aldehydes and ketones, and as a result, both classes of compounds react similarly with many reagents. 2,4-Dinitrophenylhydrazine is commonly used to test for both types of compounds. However a distinguishing behavior of aldehydes is their reaction with mild oxidizing agents which oxidize them to carboxylic acids while ketones, which are more difficult to oxidize, remain unchanged.

Several laboratory tests that distinguish between aldehydes and ketones, therefore, take advantage of this difference in behavior towards oxidants. One of these is Tollens' silver mirror test, in which a silver ammonia complex ion is reduced, by aldehydes, to metallic silver. Fehling's and Benedict's solutions are also distinguishing reagents where the Cu(II) ion, complexed to tartarate or citrate respectively, is reduced to red cuprous oxide (Cu2O) by aldehydes but not ketones.

Carbonyl compounds (aldehydes and ketones) are conveniently identified through a number of easily prepared derivatives. These include oximes, phenylhdrazones, 2,4-dinitrophenylhydrazones and semicarbazones. These derivatives are ideal because they are easily purified, crystalline solids with sharp melting points. The mechanism of formation of these closely related derivatives involves a typical nucleophilic addition at the carbonyl carbon followed by elimination of a water molecule.

S. No.	Test	Observation
i.	**2,4-DNP test for Carbonyl group (aldehydes & ketones)**  2,4-Dinitrophenyl hydrazone	
	Add a solution of 1 or 2 drops of the sample in 2 mL methanol/ethanol to 3 mL of 2,4-dinitrophenylhydrazine reagent. Shake vigorously, and, if no precipitate forms immediately, allow the solution to stand for 15 minutes.	Yellow/red ppt (dinitrophenyl-hydrazone)

ii.	**Sodium bisulfite addition complex (aldehydes & ketones)**  	
	Place 1 mL of sodium bisulfite reagent in a test tube and add 0.3 mL of the sample. Stopper the test tube and shake vigorously.	A precipitate is a formed.
iii.	**<u>Test for Aldehydes</u>** **Schiff's test**  COLOURLESS PINK COLOUR	
	To sample, add few drops of Schiff reagent and shake well. A deep red or violet colour or pink colour indicates the presence of aldehyde. **Note:** ***Amines give false positive test.***	Pink/violet colour
iv.	**Tollen's Test** $R\text{-}CHO + 2[Ag(NH_3)_2]OH \longrightarrow R\text{-}COONH_4 + 2NH_3 + H_2O + 2Ag$	
	Add one drop or a few crystals of sample to 1 mL of freshly prepared Tollen`s reagent. Gentle heating can be employed if no reaction is immediately observed.	Formation of silver mirror or a black precipitate is a positive test.
v.	**Fehling solution test** $CH_3CHO + 2CuO \longrightarrow CH_3COOH + Cu_2O$	
	Mix equal quantities of Fehling solution A and B in a test tube. Add 3 drops of the sample. Place the tube in a water-bath at	Brown red precipitate is a

	60° C and heat for 15 min.	positive test.
vi.	**<u>Test for Ketones</u>** **2,4-Dinitrobenzene test**  	
	To sample solution in test tube add a pinch of 2,4-Dinitrobenzene and NaOH a violet to pink colour formation indicates ketone.	Pink/violet colour
vii.	**Sodium Nitoprusside Test** $CH_3COCH_3 + NaOH \longrightarrow CH_3COCH_2^- + [Fe(CN)_5NO]^{--}$ $\longrightarrow [Fe(CN)_5NOCH_2COCH_3]^{--}$	
	To sample solution in test tube add solution of sodium nitroprusside and NaOH a violet to pink colour formation indicates ketone.	Violet colour
viii.	**Iodoform test** $RCOCH_3 + 3I_2 + 4NaOH \longrightarrow RCOONa + 3NaI + 3H_2O + CHI_3$ (Iodoform)	
	Dissolve 5 drops or 50 mg of the sample in 2 mL of water. Add 2 mL of NaOH (3M) and then slowly add 3 mL of iodine solution. Shake vigorously.	Formation of solid iodoform (yellow)

Aldehydes (RCHO), (ArCHO)

Formaldehyde

HCHO

Physical properties:

Molecular formula is CH_2O, colorless liquid, it has characteristic pungent odor.

Chemical properties:

1- Schiff`s reagent test- Add 2 mL of Schiff s reagent to 2 mL cold aldehyde solution shake vigorously and allow standing for two minutes – a deep – violet color which indicates the presence of aldehydic group.

2- In dry test tube put 2 mL of formaldehyde with few crystals of resorcinol then add 2 mL conc. H_2SO_4 carefully from the side of the tube. The red ring formed and white ppt. in aqueous layer turns to violet red.

3- It reduces Fehling`s reagent- Add 1mL of formaldehyde solution to Fehling`s solution (1mL of Fehling A + 1mL of Fehling B) and heating the solution notice the blue color convert to red color

4- Add 1% phenyl hydrazine + 2 mL of formaldehyde + few drops of sodium nitro prusside solution in excess of NaOH. Blue color will appear then turns to green then red then brown.

5- Add diluted formaldehyde solution + 1% phenyl hydrazine + 5% 2 mL pot. ferricyanide + conc. HCl- it gives a rose red color.

6- It gives 2, 4-dinitrophenyl hydrazine m.p 166 °C.

Acetaldehyde

CH_3CHO

Physical properties:

Molecular formula is C_2H_4O, colorless liquid, it has pungent, fruity odor, b.p 20 °C, miscible with water, alcohol and ether.

Chemical properties:

1- Give violet color with chiff s reagent.

2- 2 mL aqueous sodium nitroprusside, 5 drops of NaOH and 2 mL of acetaldehyde gives a deep win red color.

3- It responds to iodoform test

4- Boiling 2 mL of solution with 2 mL (20%) KOH give yellow ppt.

5- It reduces Fehling`s solution and Tollen`s reagents.

6- Formed white crystals by reaction with sodium bisulphate.

7- It gives 2,4- dinitrophenyl hydrazone, m.p 168 °C.

Benzaldehyde

Physical properties:

Molecular formula C_7H_6O, colorless liquid, immiscible with water.

Chemical properties:

1- It is gives Schiff s reagent test.

2- It reduces Fehling`s solution reagents

3- It gives violet color with $FeCl_3$.

4- It decolorizes alkaline $KMnO_4$ solution by heating, and then acidification with HCl, salicylic acid is formed as white ppt.

5- Cannizaro reaction- Boil 1 mL of benzaldehyde with 2 mL of NaOH and cool the solution then acidify with HCl, white precipitate of benzoic acid is formed.

Ketones (RCOR), (ArCOR)

Acetone

Physical properties:

Molecular formula is C_3H_6O, colorless liquid, it has characteristic pleasant smell, miscible with water, alcohol, and ether.

Chemical properties:

1- Colours test.

- To 1 mL of sodium nitroprusside with 0.5 mL of NaOH add 1 mL of acetone and notice appearance of red colour.

- Add 1 mL of acetone to 1 mL of sodium nitroprusside with 0.5 mL of pyridine and notice appearance of blue colour.

- To 1 mL of acetone with 0.5 mL of NaOH add 0.5 gm of m-dinitrobenzene and notice the appearance of red colour.

2- Iodoform test. Add 3-4 drops of iodine solution and then NaOH solution drop by drop to the sample and warm the brown color of iodine disappear and a yellow ppt. is formed.

3- Ding`s test. Addition of 2 mL of acetone and 2 mL of acidic solution of mercury sulphate then heating on water bath produce heavy white precipitate.

4- To 2 mL of standard sodium bisulphite add few drops of acetone. White crystals are formed.

5- Oxidation test. Addition of 1 mL of acetone to 2 mL of acidic $KMnO_4$ solution and heating lead to disappear of violet color of permanganate.

6- 2,4-Dinitrophenyl hydrazine test. To 3 mL of alcoholic solution of 2,4-dinitrophenylhydrazine add 1 mL of acetone and heating the mixture in water bath and notice formation of yellow precipitate.

Acetophenone

Physical properties:

Molecular formula is C_8H_8O, colorless liquid, it has characteristic sweet smell. It is sparingly soluble in water, soluble in alcohol, ether, and chloroform.

Chemical properties

1- It is responds to iodoform test.

2- Orange color is produced on dissolving in conc. H_2SO_4.

3- With 2,4-dinitrophenyl hydrazine, it gives phenyl hydrazone of acetophenone having m.p 250 ^oC.

Benzophenone

Physical properties:

Molecular formula is $C_{13}H_{10}O$, m.p 48.5 °C, colorless solid, insoluble in water, but soluble in alcohol and ether.

Chemical properties

1- Dissolve benzophenone in conc. H_2SO_4, it gives yellow solution.

2- Fusing the substance with sodium metal produces blue color.

3- It gives phenyl hydrazone with 2,4-dinitro phenyl hydrazine.

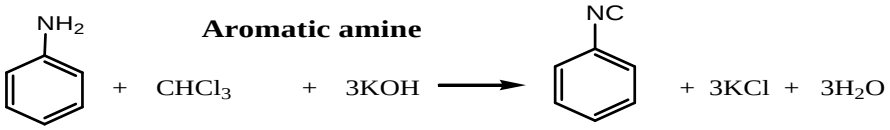
4- Boiling the solid with NaOH gives oil drops.

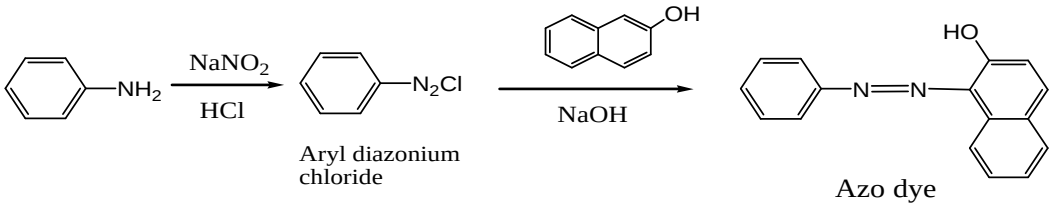
12. FUNCTIONAL GROUP TEST (TEST FOR PRIMARY, SECONDARY AND TERTIARY AMINES)

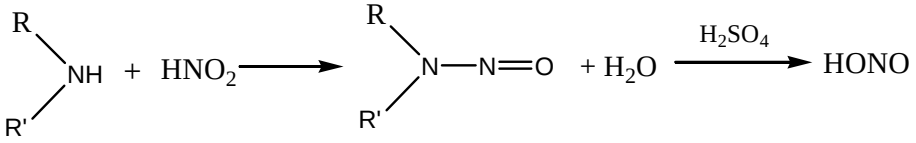
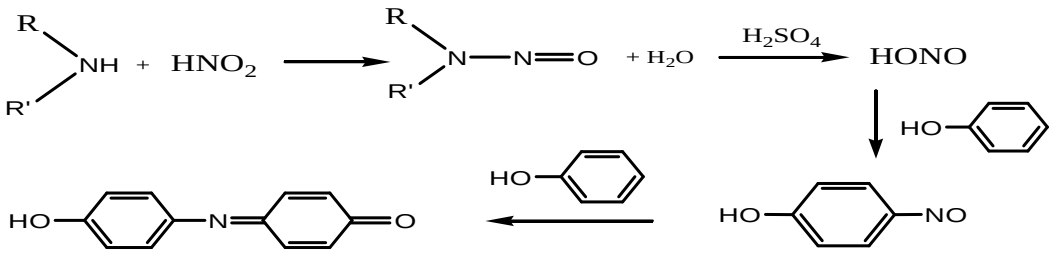
Amines are basic organic compounds that contain nitrogen and may be considered as derivatives of ammonia. They are classified as primary, secondary, or tertiary, depending on the number of alkyl groups attached to the nitrogen atom. Like ammonia, amines are electron donors and behave both as bases and as nucleophiles. Alkyl amines are considerably stronger bases than aryl amines. The diminished basicity of aryl amines is attributed to the appreciable overlap of the lone pair with the pi-electrones of the benzene ring making them less available for donation.

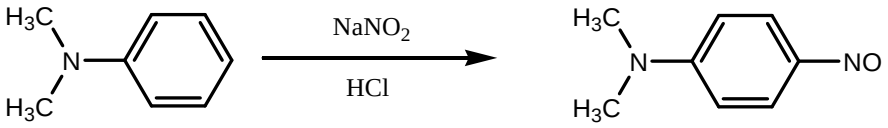
Amines form hydrogen bonds but not as strongly as alcohols. Nitrogen is less electronegative than oxygen. Tertiary amines cannot hydrogen bond to each other. Amines have boiling points between alkanes and alcohols. Tertiary amines boil lower then 10 or 20 of similar molecular weight. All amines can form hydrogen bonds with water. Amines up to 6 carbons long are water soluble due to this hydrogen bonding. Water solubility decreases as the length of the hydrocarbon portion of the molecule increases.

Amines are classified by the number of carbons directly bonded to the nitrogen atom: A primary amine has one (RNH_2 = 1°); A secondary amine has two (R_2NH = 2°); A tertiary amine has three (R_3N = 3°).

S. No.	Test	Observation
i.	**<u>Test for Primary amines (1° amines)</u>** **Carbylamine test (Isocyanide test):** This test is used to distinguish 1° amines from 2° and 3° amines. It is given by both 1° aliphatic and 1° aromatic amines. **Aliphatic amine** $RNH_2 + CHCl_3 + 3KOH \longrightarrow R\text{-}NC + 3KCl + 3H_2O$ **Aromatic amine**  	
	To sample, add alcoholic KOH and 2 drops of chloroform and heat gently. An intolerable offensive smell (phenyl cyanide) indicates primary amine.	Offensive smell
ii.	**Azodye test**	



	Dissolve a little sample in 2 mL of conc. HCl, dilute with water & cool in ice. Dissolve sodium nitrite in water & add the solution dropwise to the cold solution nitrite in water and cool again. Add this solution drop wise to the cold solution of alkaline phenol (β naphthol).	Red colour dye
iii.	**<u>Test for Secondary amines (2^o amines)</u>** Secondary amines do not show carbylamine and azodye test. They can be identified by nitrous acid reaction and Liebermann Nitroso reaction. **Nitrous acid reaction (for aliphatic secondary amines)**  	
	To organic Sample add 1mL dil. HCl and cool under tap water. Add 2mL $NaNO_2$ solution and shake gradually. Separation of yellow oil indicates aliphatic secondary amine.	Yellow oil
iv.	**Liebermann Nitroso reaction: (for aromatic secondary amines)** This test is given only by 2^o amines (both aliphatic and aromatic). 2^o Amine is converted into nitroso amine by treating the amine with HNO_2. On warming with phenol and conc. H_2SO_4, brown or red colour is formed at first, which changes to blue then to green. Colour changes to red on dilution and further to greenish blue or violet on treatment with alkali.  	

	Take organic sample add 1mL dilute HCl and cool under tap water. Add $NaNO_2$ solution and shake gradually, yellow oil separates. Add sulfuric acid and phenol, warm gently for few seconds green to deep blue colour is obtained. Add water colour changes to red which turns blue again on addition of NaOH.	Green to deep blue colour
v.	**<u>Test for Tertiary amines (3^o amines)</u>** Tertiary amines do not show carbylamine and azo-dye test. They can be identified by colour reaction with sodium nitrite. **Sodium nitrite test**  *p*-Nitroso dimetyl aniline	
	Take organic sample and add 1mL dilute HCl, and cool under tap water. Add $NaNO_2$ solution and shake gradually. **i.** If no reaction happens it indicates aliphatic tertiary amine. **ii.** If green or brown colour appears it indicates aromatic tertiary amine.	

Aromatic amines

Aniline

Physical properties: Molecular formula is C_6H_7N, colorless to yellow liquid, b.p.184° C, sparingly soluble in water, when aniline is exposed to air it is darkness in color and become black.

Chemical properties:

1- Azo dye test: Dissolve aniline with 2.5 mL of conc. HCl, cool and dilute with about 3 mL H_2O, cool in ice bath and add with shaking 2 mL of diluted $NaNO_2$ solution. Then cool and add this diazotized solution to cooled solution of β-naphthol dissolved in 10 % NaOH, a scarlet ppt. is appeared.

2- Add 2 drops of aniline to dil. H_2SO_4, then add $K_2Cr_2O_7$solution, a green blue or black ppt. is formed due to oxidation.

3- With $FeCl_3$, solution of aniline gives pale green color.

4- Shake 2 drops of aniline with 5 mL of water; add few drops of NaOCl solution, a purple color is formed which soon turns brown.

o-Toludine

Physical properties:

Molecular formula is C_7H_9N, liquid, b.p. 199° C, sparingly soluble in water but soluble in mineral acids.

Chemical properties:

1- In azo dye test: it gives orange or red ppt.

2- With $FeCl_3$, gives greenish color.

3- Shake 2 drops of *o*-toludine with 5 mL of alcohol; add few drops of NaOCl solution, a brown color is formed.

p-Toludine

Physical properties:

Molecular formula is C_7H_9N, solid, mp.45° C, sparingly soluble in water but soluble in mineral acids.

Chemical properties:

1- In azodye test: it gives orange or red ppt.

2- With $FeCl_3$, gives brown color.

3- Shake 2 drops of *p*-toludine with 5 mL of alcohol

4- Add few drops of NaOCl solution, a yellow color is formed.

α – Naphthyl amine

Physical properties:

Molecular formula is $C_{10}H_9N$, colorless solid, when exposed to air it becomes violet, mp. 48-50 °C, sparingly soluble in water and soluble in mineral acids.

Chemical properties:

1- In azo dye test: it gives orange or red ppt.

2- With $FeCl_3$, gives blue color.

3- conc. HCl + H_2O + $FeSO_4$, it gives green ppt.

Aliphatic amines

Glycine

Physical properties:

Molecular formula is $C_2H_5NO_2$, colorless crystalline solid, soluble in water; it exhibits acidic and basic characters.

Chemical reactions:

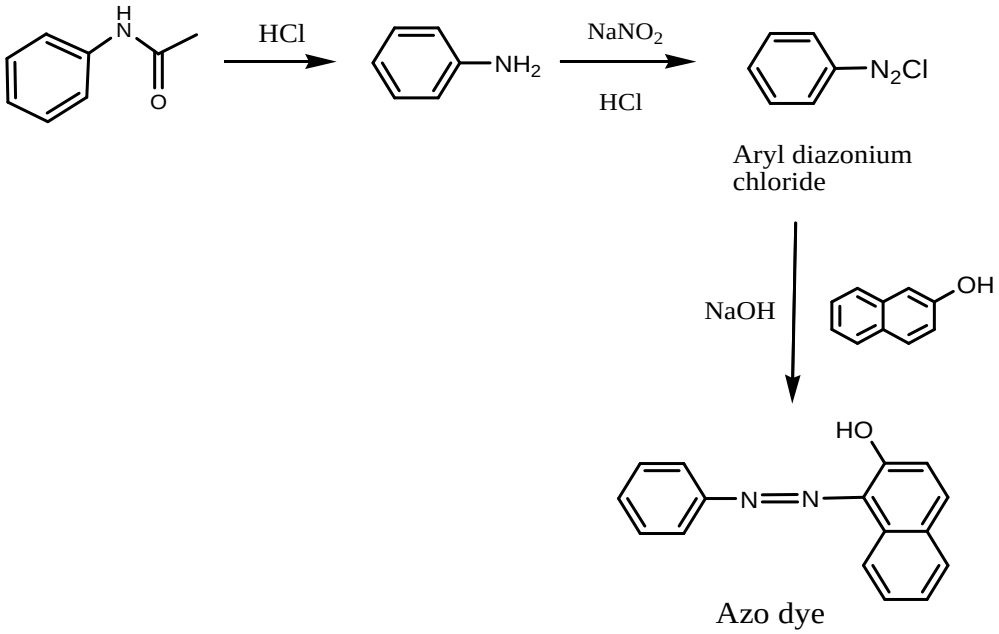
1- It does not give nitration test.

2- Gives weak acidity test.

3- Solution + copper acetate, it gives blue color.

4- Solution + $FeCl_3$, it gives red color.

13. FUNCTIONAL GROUP TEST (TEST FOR ANILIDES)

S. No.	Test	Observation
i.	**Carbylamine test (Isocyanide test)**	
	To sample add alcoholic KOH and 2 drops of chloroform in a test tube and heat gently.	An intolerable offensive smell
ii.	**Tafels Test**	
	To sample add conc. H_2SO_4 and shake add pinch of potassium dichromate. A red or violet colour is obtained. Leave for few minutes the colour changes to green.	A red or violet colour
iii.	**Hydrolysis test** 	
	To sample, add dil. HCl, boil and cool. Add $NaNO_2$ solution, cool again & add cold alkaline β-naphthol solution.	An orange red dye is produced.

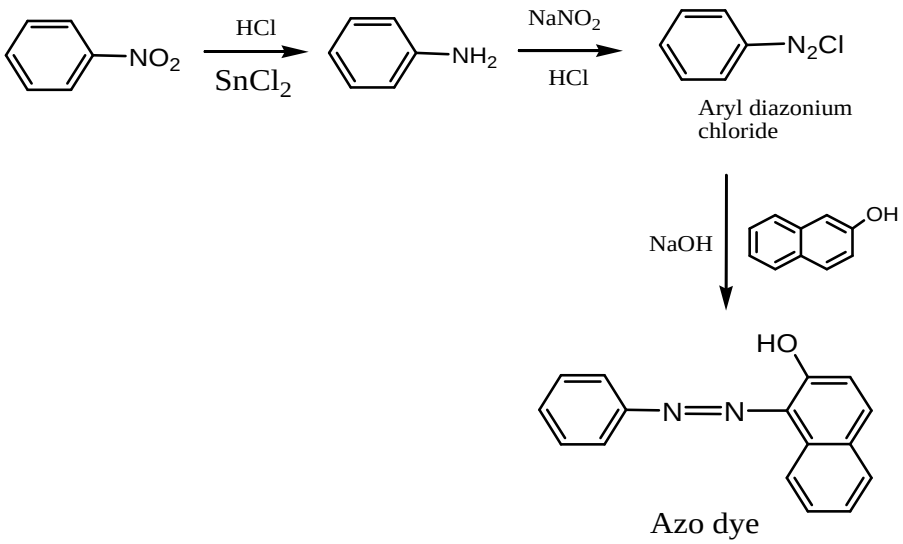
14. FUNCTIONAL GROUP TEST (TEST FOR AMIDES)

Amides are generally derived from amines. They also give some chemical reactions of amines. Variety of medicinal compounds has amide linkage in their structure. The amide linkage -CONH- interacts with different enzymes and bio-molecules in the body thereby affecting the biochemical processes.

S. No.	Test	Observation
i.	**Sodium hydroxide test** $R\text{-}CONH_2 + NaOH \longrightarrow R\text{-}COONa + NH_3$	
	To sample, add 1 mL of aqueous NaOH and heat it. Keep the moist red litmus on the mouth of test tube.	Smell of ammonia Litmus turns blue

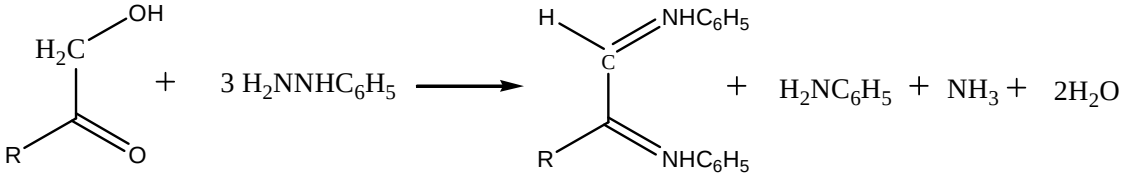
15. FUNCTIONAL GROUP TEST (TEST FOR NITROSO GROUP)

Presence of nitroso group in compounds may result in potential bioactivity. Several drugs have nitroso linkage in their structure.

S. No.	Test	Observation
i.	**Mullikens Test** $RNO_2 + 4[H] + Zn + NH_4Cl \longrightarrow RNHOH + H_2O$ $RNHOH + 2Ag(NH_3)_2OH \longrightarrow RNO + 2H_2O + 2Ag + 4NH_3$	
	Take sample in a test tube, add alcohol and NH_4Cl solution, add a pinch of zinc dust, boil, cool and filter. To the filtrate add Tollen`s reagent.	Grey/black silver mirror is produced.
ii.	**Azodye test**  	
	To the sample, add conc. HCl and water and stannous chloride. Heat on water bath for 10-15 min. Filter and cool to 0-5°C, to the filtrate add $NaNO_2$ solution dropwise and cool again. Add this solution drop wise to the cold solution of alkaline β-naphthol.	A red coloured dye is obtained

16. FUNCTIONAL GROUP TEST (TEST FOR CARBOHYDRATES)

Carbohydrates may be classified as reducing and non-reducing sugars. There are several classifications for carbohydrates. Table sugar is sucrose which is a disaccharide.

S. No.	Test	Observation
i.	***Molisch's Test*** Place 2 mL of the carbohydrate solution in a test tube, add 1 drop of Molisch's reagent (10% α-naphthol in ethanol). Add 1-2 mL of conc. H_2SO_4 down the side of the test tube, so that it forms a layer at the bottom of the tube.	Violet colour ring at the junction between the two layers
ii.	**Fehling's test or Benedict's test** To 1 mL of Fehling's solution A (aqueous solution of $CuSO_4$) add 1 mL of Fehling solution B (solution of sodium potassium tartrate). Add 2 mL of the sugar solution, mix well and boil.	A red precipitate of cuprous oxide is obtained.
$CHO(CHOH)_4CH_2OH + 2Cu^{2+} + 5OH^- \rightarrow COOH(CHOH)_4CH_2OH + Cu_2O + 3H_2O$ Glucose — Gluconic acid		
iii.	**Barfoed's test** To 1-2 mL of Barfoed's reagent, add an equal volume of sugar solution. Boil for 5 min in a water bath and allow to stand for a while.	A brick-red cuprous oxide precipitate is formed.
	Tollen's test sample + Tollen's reagent (amm. silver nitrate solution). Heat on water bath. A silver mirror is obtained the walls of the test tube	
$CHO(CHOH)_4CH_2OH + 2[Ag(NH_3)_2]^+ + 3OH^- \rightarrow COOH(CHOH)_4CH_2OH + 4NH_3 + 2Ag\downarrow + 2H_2O$ Glucose — Gluconic acid		
iv.	**Osazone test** Place 0.2 gm of the sample in a test tube and add 0.4 g of phenylhydrazine hydrochloride, 0.6 gm of crystallized sodium acetate, and 4 mL of distilled water. Place the test tube in a beaker of boiling water. Note the time that the test tube was immersed and the time of the precipitation.	Time taken for precipitate formation: Fructose- 2 min; glucose- 4-5 min; sucrose- 30 min
 $+ \; 3\,H_2NNHC_6H_5 \rightarrow$ $+ \; H_2NC_6H_5 + NH_3 + 2H_2O$		

17. IDENTIFICATION AND REACTIONS OF HYDROCARBONS

Hydrocarbons, compounds which contain only carbon and hydrogen, can be classified into several types, depending on their structure. Aliphatic hydrocarbons are divided into three classes: alkanes have only single bond. Aliphatic hydrocarbons are divided into three classes: alkanes have only single bonds, and are said to be saturated; alkenes and alkynes have carbon-carbon double or triple bonds, and are said to be unsaturated.

Aromatic hydrocarbons are cyclic compounds whose structure is related to that of benzene, with six -electrons in a six-member ring. Aliphatic Hydrocarbons such as Alkanes are relatively inert to chemical oxidizing agents such as neutral or alkaline permanganate, where alkenes are readily oxidized at room temperature. Hydrocarbons are divided into two categories. Solids and liquids some examples of solid hydrocarbons are (naphthalene, Anthracene) and liquid hydrocarbons are (toluene, benzene, n-hexane).

TESTS:-

All tests should be carried out in dry test tubes, and observations should be recorded on the report sheet as each experiment is performed.

(A) Solubility of hydrocarbons

Add about 2 mL of water in a small test tube and add 2 or 3 drops of the hydrocarbon to be tested. Shake the mixture to determine whether the hydrocarbon is soluble (a colorless second layer may be hard to see). Record the results.

(B) Bromine Test

In a small test tube, add 1 mL of hydrocarbon to (3-4 mL) of 2% bromine in carbon tetra chloride. Shake well and observe after two or three minutes.

If the bromine is not decolorized then perform the following test-

Prepare a second similar tube and place the tube in your laboratory locker and the other in bright sunlight. Allow both tubes to stand for ten to fifteen minutes and compare them. Observe the color of each tube, and whether or not hydrogen bromide was evolved and record the results.

(C) Aqueous Potassium Permanganate (Baeyer's Test)

In a small test tube, add 1 mL of hydrocarbon to a mixture of 3 mL of dilute potassium permanganate solution (0.5 % $KMnO_4$ solution) and 3 mL of dilute sodium carbonate solution (10% Na_2CO_3 solution) and shake the tube for 1-2 minutes, and note the results. If the colure of $KMnO_4$ changes it is cyclohexene, and if colorless then it is alkane or aromatic hydrocarbon.

(D) Sulfuric Acid Test

In a small test tube add 1 mL of hydrocarbon, cautiously and with gentle shaking to about 3 mL of concentrated sulfuric acid. Shake the tubes well and note the results. Observe whether heat evolved and whether the hydrocarbon dissolves. Discard the contents by pouring them into a beaker containing at least 50 mL of water. Although alkanes are inert to cold, concentrated sulfuric acid, alkenes react by addition. The product alkyl hydrogen sulfate is soluble in concentrated sulfuric acid.

DIFFERENTIATION BETWEEN ALKANES AND AROMATIC HYDROCARBONS

Reaction of hydrocarbons with aluminum chloride

The reaction of aromatic hydrocarbons with aluminum chloride ($AlCl_3$) and chloroform ($CHCl_3$) to produce a brightly colored compound is known as a Friedel-Crafts reaction.

1- Place 2 mL of $CHCl_3$ into clean and dry small test tube.

2- Add two drops of the unknown hydrocarbon to test tube and gently swirl the tube.

3- Add 0.5 gm of $AlCl_3$ so that some of the solid strikes the side of the tube wall that is moisten with the unknown hydrocarbon

If a brightly colored compound is obtained then it is aromatic hydrocarbon. If no brightly colored compound then it is an alkane (n-hexane).

BENZENE

C_6H_6, Colorless liquid, b.p 80 ºC, immiscible with water.

Reactions of benzene

1- Freezing test:

Place 1 mL of benzene in dry test tube then cool it in ice, it is solidified to a colorless crystalline solid, which melts to a liquid when the tube is warmed by hand.

2- Nitration test:

Add 1 mL of benzene gradually to a mixture of concentrated nitric acid and sulphuric acid. Shake the mixture, it becomes hot (cool if necessary).pour the mixture into a beaker, which contain 50 mL cold water yellow oil of nitrobenzene is separated at the bottom of the beaker.

3- Sulfonation reaction:

Add 1 mL of benzene to 5 mL of sulphuric acid and heat the mixture on water bath using a condenser. Observe that benzene disappear gradually. Cool then pour the liquid into cold water, a homogeneous solution of benzene sulphonic acid is obtained, which is water – soluble.

4- Bromination test:

Add 2 mL of liquid brome to 1 mL of benzene in test tube and then add small species of iron to the mixture and notice the disappearance of liquid brome color. Then the mixture is poured onto ice water and notice formation of oily layer from bromobenzene.

TOLUENE

C_7H_8, Clear liquid, b.p 110 ºC, water insoluble liquid with the typical smell of paint Thinners.

Reactions of Toluene

1 - Freezing test:

Toluene does not solidify readily like benzene and temperature must be reduced to –93 ºC to form solid.

2 –Oxidation test:

Add 2 mL of toluene to solution of (potassium dichromate in conc. Sulphuric acid) and heat the mixture gently under reflux for 3 hours. Remove the excess of dichromate by passing sulphur dioxide gas in the solution then neutralize it with a saturated solution of sodium carbonate. Concentrate the alkaline solution by evaporation then acidify it with dil.H_2SO_4. Filter the formed benzoic acid and determine its melting point.

3- Nitration test:

Add 1 mL of toluene gradually to a mixture of concentrated nitric acid and sulphuric acid. Shake the mixture, it becomes hot. Heat gently then cool and pour the mixture into a beaker, which contain 50 mL cold water yellow heavy oil of nitro toluene is separated at the bottom of the beaker.

NAPHTHALENE

$C_{10}H_8$, White solid crystals, Bp: 80.26 °C, insoluble in water but soluble in acetone.

Reactions of naphthalene:

1- Nitration test:

Dissolve by heating 0.5 gm of naphthalene in 3mL of glacial acetic acid. Then cool the solution. Add 1 mL of conc. Nitric acid and heat the mixture gently for one minute. Cool and pour the solution into baker containing cold water. Yellow solid of nitro naphthalene is separated.

2- Sulfonation test:

Add conc. Sulphuric acid gradually to naphthalene at 160 ºC and keep the temperature constant at this temperature for 10 minutes. Leave the mixture aside for some times to cool, then pour it carefully into a beaker containing cold water, β-naphthalene sulphonic acid separated as solid hydrate, recrystallize by adding half its weight of water at 70 C then adding 1/6 its weight of conc. Hydrochloric acid salt of β-naphthalene sulphonic acid is separated.

3- Picrate formation:

Add concentrated solution of (picric acid in acetone) to concentrated solution of naphthalene in acetone and shake the mixture well then put it aside for some times. Yellow needles of naphthalene picrate are formed.

ANTHRACENE

$C_{14}H_{10}$, Colorless liquid, b.p: 218 ºC, insoluble in water but soluble in acetone.

Reactions of anthracene:

1 – Oxidation reaction:

Dissolve 1 gm of anthracene in acetic acid then add 3 mL of sulfuric acid. Add 4 mL of solution of potassium dichromate. Cooling the mixture and poured onto cold water lead to formation of yellow precipitate of anthraquinone.

2- Picrate formation:

Add concentrated solution of (picric acid in acetone) to a hot concentrated solution of anthracene in acetone. Heat the red solution on the water bath for few seconds then pour it in evaporating dish and leave it for some time. Red crystals of anthracene picrate appear.

18. DERIVATIVES PREPARATION

The preliminary examination and group identification tests indicate the particular class (functional group) to which an unknown organic compound may belong. Identification and characterization of the structures of unknown substances are an important part of organic chemistry. Further characterisation and identification depends on the selection and preparation of a suitable solid derivative and accurate determination of its melting point. Conversion of the unknown to a solid derivative of known melting point will often provide final confirmation of structure.

Derivatives preparation of some important classes of organic compounds is given below.

1. CARBOXYLIC ACIDS:

a) Amide derivative:

Place 0.5-1 gm of dry into a flask fitted with a reflux condenser, add 2.5-5 mL of redistilled thionyl chloride and reflux gently for 30 minutes remove the excess of thionyl chloride by distillation. Treat the acid chloride with excess of ammonia solution and warm for a few min. If no solid separates on cooling, evaporate to dryness on a water bath. Recrystallize the crude amide from water or dil. ethanol and determine the melting point.

$$RCOOH + SOCl_2 \longrightarrow RCOCl \xrightarrow[NH_3]{\text{Excess}} RCONH_2$$

b) Benzylamide derivative:

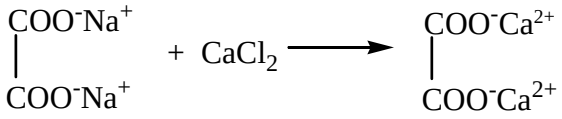
Many acids when heated directly with benzylamine in the presence of NH_4Cl as a catalyst give the corresponding *N*-benzylamides.

Heat together under reflux 0.5 gm of compound, 3mL of benzylamine and 0.15 gm of NH_4Cl for 30-45 min. Cool, shake with about 10 mL of water and filter off the solid. Re-crystallise from ethanol and determine the melting point.

$$RCOOH + H_2N\text{-}CH_2.C_6H_5 \xrightarrow{NH_4Cl} RCONHCH_2\text{-}C_6H_5$$

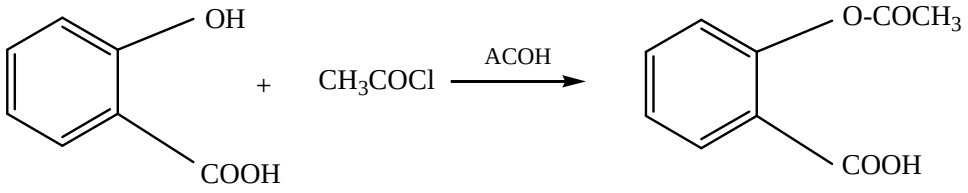
- In case of oxalic acid: urea/ calcium oxalate:

Add $CaCl_2$ / urea solution to a neutral solution of an oxalate a white precipitate of cal. Oxalate is formed which is filtered off.



❖ In case of salicylic acid: acetyl derivative:

Boil under reflux 1gm of compound + 4 mL of acetic anhydride or acetyl chloride- acetic acid mixture (equal volumes) for 10 min. Pour it in to cold water. Filter off the acetyl derivative (acetyl salicylic acid) and determine the melting point.

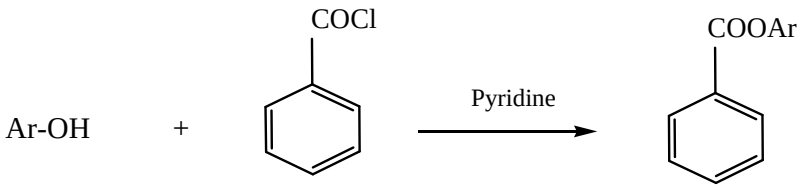


2. PHENOLS:

a) Benzoate derivatives:

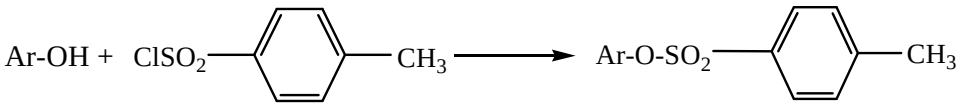
Suspend 1 gm (or 1 mL) of the substance in 20 mL of 5% NaOH solution in a well- stoppered flask, add 2 mL of benzoyl chloride drop wise at a time, with constant shaking and cooling in water (if necessary). Shake vigorously for 5-10 min until the odour of benzoyl chloride has disappeared. Filter off the solid benzoyl derivative, wash it with a little cold water and re-crystallise it from ethanol and determine the melting point.

Alternatively, dissolve 1 gm (or 1 mL) of the substance in 3 mL of dry pyridine and add 0.5-1 mL of benzoyl chloride. After initial reaction has subsided, warm the mixture over a small flame for a min or two and pour, with vigorous stirring, into 10-15 mL of water. Allow the ppt. to settle, decant the supernatant liquid, stir the residue thoroughly with 5-10 mL of sodium carbonate solution, filter and Recrystallize from ethanol and determine the melting point.



b) Toluene-*p*-sulphonates:

Toluene-*p*-sulphonyl chloride reacts readily with phenols to yield toluene-*p*-sulphonates.



Mix 1 gm (or 1 mL) of the substance with 2.5 mL of dry pyridine, add 2 gm of toluene-*p*-sulphonyl chloride and heat on a water bath for 15 min. Pour into 25 mL of cold water and stir until the oil solidifies. Filter, wash with cold dil. HCl (to remove pyridine), with cold dil. NaOH (to remove any phenol present), and then with cold water. Recrystallize from methanol or ethanol and determine the melting point.

3. ALDEHYDES AND KETONES:

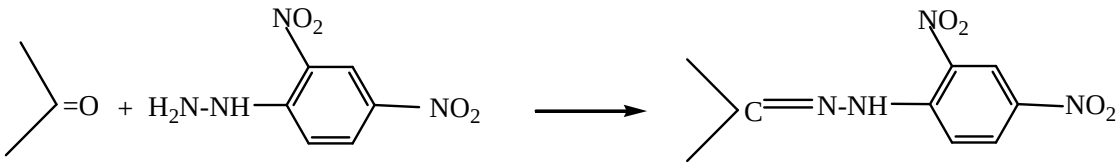
a) 2,4-Dinitro-phenyl (2,4-DNP) derivatives:

Warm 2,4-Dinitrophenyl hydrazine (2,4-DNP) solution and add the solution of carbonyl compound in a small volume in methanol or ether. If no solid separates with in 10min, dilute the solution carefully with 1M H_2SO_4. Collect the solid by filtration and wash it with a little aq. methanol. Recrystallize it from ethanol and determine the melting point.

(OR)

Alternatively, to the clear solution obtained by warming 0.5 gm of 2,4-DNP, 1 mL of con. HCl and 8-10 mL of ethanol, add 0.25 gm of the carbonyl compound and heat to boiling. Allow, cooling to room temperature, filtering off the solid, recrystallize it from ethanol and determine the melting point.

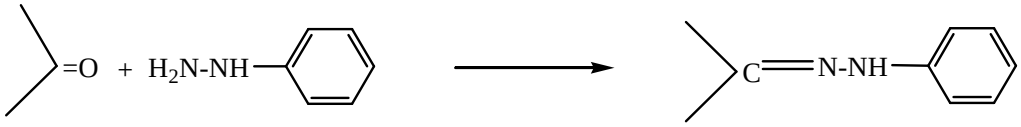
Commonly for the preparation of 2,4-DNP derivatives, dissolve the carbonyl compound (0.5 gm) in 5 mL of ethanol, and add the calculated volume of the reagent. If a ppt. does not form immediately, dilute with a little water. Collect the derivative and determine the melting point.



b) Phenyl hydrazones:

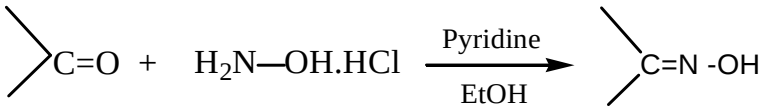
Dissolve 0.5 gm of phenyl hydrazine hydrochloride and 0.8 gm of sodium acetate in 5 mL of water, and add a solution of 0.2-0.4 gm of carbonyl compound in a little ethanol. Shake the mixture until a clear solution is obtained and add a little more ethanol, if necessary. Warm on a

water bath for 10-15 min and cool. Filter off the crystalline derivative and recrystallize it from dil ethanol or water and determine the melting point.



c) Oximes: (Water insoluble aldehydes or ketones)

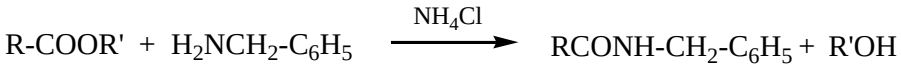
Reflux a mixture of 0.5 gm of carbonyl compound, 0.5 gm of hydroxylamine hydrochloride, 5 mL of ethanol and 0.5 mL of pyridine on water bath for 30-45 min. Remove the ethanol by evaporation on water bath. Add 5 mL of water to the cooled residue. Cool in ice bath and stir until the oxime crystallizes. Filter off the solid, wash it with a little water and dry in desiccators. Recrystallize from ethanol and determine the melting point.



4) ESTERS:

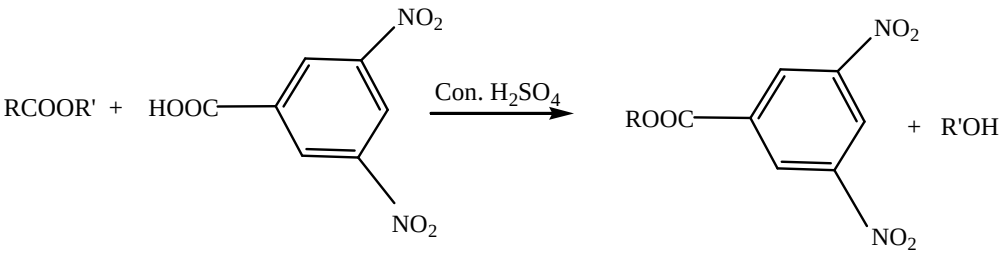
a) Benzylamide derivative:

Heat together under reflux 1 gm of compound, 3 mL of benzylamine and 0.15 gm of NH_4Cl for 30-45 min. Cool, shake with about 10mL of water and filter off the solid. Recrystallize from ethanol and determine the melting point.



b) Ester exchange reaction:

To about 1 mL of ester add about 0.5 gm of 3,5-dinitro benzoic acid and 0.25 mL of con. H_2SO_4. Heat the mixture gently under reflux for 5 min. Add very carefully about 10 mL of water, make alkaline with NaOH and filter off the solid, dry, and determine the melting point.

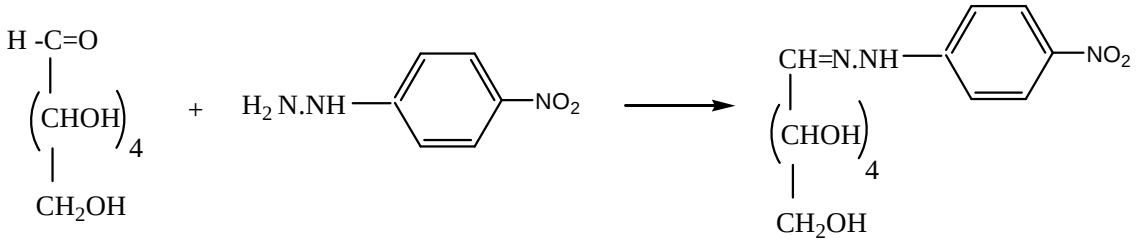


5) CARBOHYDRATES:

a) Osazone formation:

b) *p*-Nitrophenyl hydrazones:

Heat the compound (sugar; glucose) with ethanol, add *p*-nitrophenyl hydrazine and heat the suspension until the reaction appears complete. The *p*-nitrophenyl hydrazones soon separates. Filter preferably after standing overnight, wash with a little cold ethanol.



6) HYDROCARBONS:

Picrate derivatives:

Are usually prepared by adding hot solution of the compound in ethanol to a cold saturated ethanolic solution of picric acid, warm and allow to cool; the derivative separate in crystalline condition. It is filtered off, washed with a little ether, dry and determine the melting point. Quantity of compound and picric acid should be equimolar.

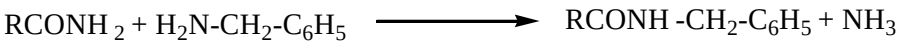
7) AMIDES:

a) Hydrolysis:

Place together about 1gm of compound and 10mL of 10% NaOH solution. Fit a reflux condenser and boil gently for about 20min. Cool the flask, add an excess of dil. H_2SO_4 and cool thoroughly. Filter off the solid and determine the melting point.

b) *N*-Benzylamide:

Heat together under reflux 1 gm of amide, 3 mL of benzylamine and 0.15 gm of NH_4Cl for 30-45 min. Cool, shake with about 10mL of water and filter off the solid and determine the melting point.



In case of urea:

Urea nitrate/oxalate: Prepare a conc. solution of urea.

a) To one portion, add few drops of conc. HNO_3, the white crystalline urea nitrate is precipitated.
b) To another portion, add conc. aq. oxalic acid solution, white crystals of urea oxalate separate.

8) AMINES:

a) Acetyl derivative:

1 mL of aniline is added to 3 mL of acetyl chloride, a vigorous reaction occurs and a solid mass is formed. Add just sufficient water (about 15 mL) to dissolve the solid completely on boiling. On cooling, crystals of acetyl derivative separate out.

$$R\text{-}NH_2 + CH_3COCl \longrightarrow RNHCOCH_3$$

b) Benzoyl derivatives:

A mixture of 1mL of amine, 15mL of 10% NaOH solution and 1.5mL of benzoyl chloride is shaken. Benzoyl derivative separates out.

$$R\text{-}NH_2 + C_6H_5COCl \xrightarrow{NaOH} RNHCOC_6H_5$$

9) ANILIDES OR SUBSTITUTED AMIDES:

a) With 10% sulphuric acid:

Reflux 1 gm of compound (eg. acetanilide) with 20 mL of 10% sulphuric acid for 1-2 h. Cool the residue, render it alkaline with 20% NaOH solution, cool and extract with ether. Distilled of the ether and examine the ether sue for an amine.

$$C_6H_5\ NH\ COCH_3 \xrightarrow{H_2SO_4} C_6H_5NH_3^+HSO_4^- + CH_3COOH \xrightarrow{NaOH/ether} C_6H_5NH_2$$

b) With 70% sulphuric acid:

Reflux 1 gm of compound (eg. benzanilide) with 10-15 mL of 70% sulphuric acid for 30 min. Allow to cool and wash down any acid, which has sublimed in to the condenser with hot water. Filter off the acid, wash it with water and determine the melting point.

10) NITRO COMPOUND:

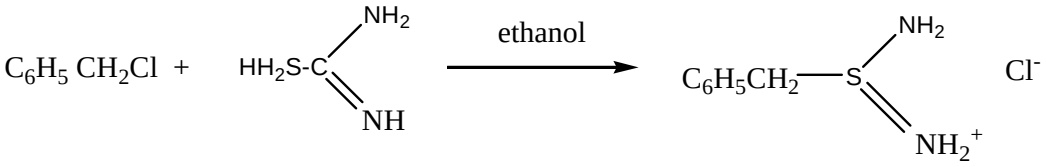
Nitro derivative:

Add about 0.5 gm of compound to 2 mL conc. sulphuric acid. Introduce 2 mL of concentrated nitric acid drop by drop with shaking after each addition. Attach a reflux condenser to the flask and heat on a cooling water bath 50 ˚C for 5 min. Pour the reaction mixture on to a beaker containing the cubes and collect the precipitated solid by filtration. Recrystallize from dil. ethanol and determine the melting point.

11) THIOAMIDE (THIOUREA):

Benzyl thiouronium chloride:

Thiourea unlike urea, readily react in the tautomeric form in the presence of suitable reagents particularly alkyl halides. Thus benzyl chloride reacts with thiourea in ethanolic solution to give benzyl thiouronium chloride.



Add 4 mL of benzyl chloride and 2.5 gm of thiourea to 5-10 mL of ethanol in a flask with a reflux condenser. Warm the mixture on the water bath with gentle shaking until the reaction occurs and effervescence subsides; then boil the mixture under reflux for 30 min. Cool the clear solution in ice water, filter of the crystalline deposit of the benzyl thiouronium chloride, wash it with ice cold ethyl acetate.

SYNTHESIS OF SOME IMPORTANT COMPOUNDS / DRUGS

In the experiments of pharmaceutical organic chemistry, synthesis is also an important part. The students should learn the basic synthesis techniques involving acetylation, halogenations, hydrolysis, nitration, diazotization, reduction, cyclization, etc. Thus, syntheses of some important drugs or compounds are also provided in the following pages.

19. SYNTHESIS OF ASPIRIN

Aspirin is also known as acetyl salicylic acid. It is a widely used drug in modern society. It is an analgesic (pain killer), a powerful antipyretic (fever-reducing) and an anti-inflammatory (swelling-reducing) substance. Salicylic acid (which is a constituent of certain plants) is itself an analgesic and was originally administered as sodium salicylate. Since salicylic acid has an irritating effect on the stomach lining, chemists sought a modification which would retain its properties while decreasing the adverse side effects. Conversion to the ester satisfied this requirement and acetylsalicylic acid (*aspirin*) proved to be as effective as sodium salicylate without the irritation typical of phenolic compounds. Aspirin gets, however, hydrolyzed to salicylic acid in the alkaline medium of the intestines.

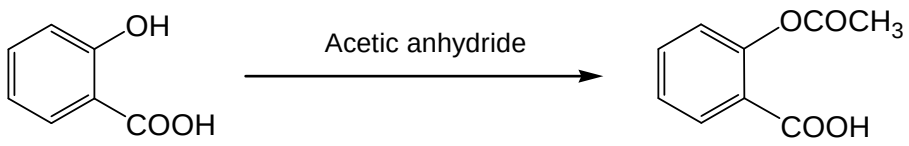
Aspirin is obtained from salicylic acid by simple acetylation with acetic anhydride or acetyl chloride in the presence of sulfuric acid as catalyst. Because of its low solubility in water (0.25 g/ 100 mL) it is isolated from the reaction mixture by precipitation with water.

PROCEDURE:-

Place 3.0 gm (0.02 mol) of salicylic acid in a 100 mL Erlenmeyer flask and add, with constant stirring, 6 mL of acetic anhydride (fume hood) followed by 1 mL of concentrated sulfuric acid. Stir the mixture gently observing the rise in temperature to 70-80 °C while the salicylic acid dissolves.

After 15 minutes the solution cools by itself to 35-45 °C and a solid mass of aspirin forms. Pour 35 mL of ice cold water over the contents of the flask to hydrolyze excess acetic anhydride and to complete the precipitation of aspirin. Collect the crude aspirin using a Buchner funnel and wash with ice-cold water. Air-dry the product, weight, and calculate the yield. Also determine its melting point.

CHEMICAL REACTION:-



Salicylic acid Aspirin

20. SYNTHESIS OF OIL OF WINTERGREEN

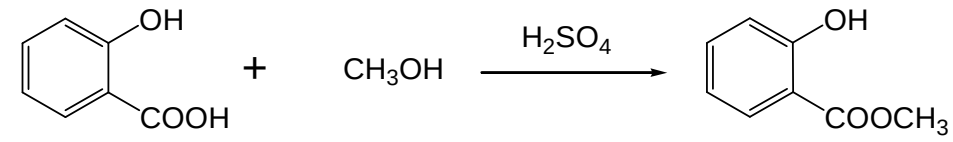
Methyl salicylate is also known as Oil of Wintergreen. It is prepared by the combination of methyl alcohol and salicylic acid in the presence of H_2SO_4. This process is known as esterification. In contrast to other salicylate, it is not used internally as it can induce vomiting, gastritis and systemic toxicity.

PROCEDURE: -

A solution of salicylic acid (17 gm) absolute methyl alcohol (30 mL) and conc. H_2SO_4 (5 mL) is refluxed for 3 hr under anhydrous condition. Excess ethanol is removed by distillation (steam bath) and the residual product is poured into water. Methyl salicylate is extracted with ether. The ether extract is washed with Na_2CO_3 solution (till free of acid) and finally with water. It is dried and distilled.

Characteristic smell of methyl salicylate (oil of Wintergreen) is obtained from the product.

CHEMICAL REACTION:-



Salicylic acid Methanol Methyl Salicylate

21. SYNTHESIS OF *p*–BROMO-ACETANILIDE BY HALOGENATION (BROMINATION)

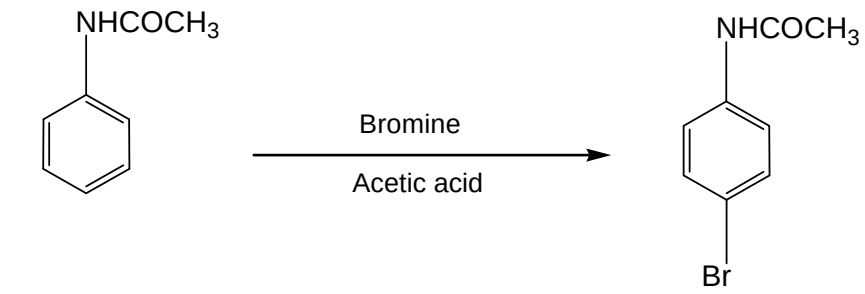
Halogenation of aromatic compound is an e.g of electrophillic aromatic substitution reaction i.e. chlorination or bromination of benzene. The major role of halogen carriers is to generate a brominium ion electrophile that eventually attacks the nucleus at the particular site of maximum electron density.

Acetanilide can be easily brominated with bromine in glacial acetic acid. In this reaction small amount of *o*–bromo acetanilide is also formed which being more soluble in alcohol can be removed during crystallization of para bromo acetanilide with the liberation of a mole of hydrogen bromide (-$NHCOCH_3$) is an ortho, para directing function. Hence the incoming bromo mostly shall yield both ortho & para isomers. The later is produced predominately up to 90% as a white solid.

PROCEDURE:-

Take 1 gm finely powdered acetanilide in 5 mL cold glacial acetic acid containing 250 mL of conical flask. Chill the content of conical flask then immediately add 6.5 mL of bromine solution from the burette drop wise very slowly with vigorous shaking in chilled content of conical flask. After mixing & shaking, allow the mixture to stand at room temperature for 25 minutes. Then pour the pale reddish orange solution which may contain some crystal of para-bromo-acetanilide into a large excess amount i.e. about 60 mL of cold water where upon the para-bromo-acetanilide will rapidly crystallize out. Stir these crystals thoroughly & wash with the water to eliminate acetic acid, unchanged bromine etc. & then filter, wash well with cold water, drain & finally Recrystallize. The melting point is about 167^o C & yield is about 1 gm. The crude product may be recrystallized from the rectified spirit either at room temperature or slightly warming it in electric water bath. The yield of pure coloured para-bromo-acetanilide is slightly reduced.

CHEMICAL REACTION:-



Acetanilide

p-Bromo-acetanilide

22. SYNTHESIS OF OXALIC ACID FROM SUCROSE

Oxalic acid is formed by the reaction between sucrose (cane sugar) and conc. HNO_3. It is a colourless compound and poisonous in nature. Its melting point is 101^oC.

PROCEDURE:-

Take 10 gm cane sugar in a round bottom flask then add 50 mL HNO_3, then heat on the water bath. After 15 min, pour the reaction mixture into a conical flask. Allow the mixture to evaporate and reduce its volume to 10 mL. Now add 20 mL ice cold water into the conical flask. When crystals are formed, filter and recrystallize.

CHEMICAL REACTION:-

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + \underset{\text{from Nitric acid}}{18[O]} \longrightarrow \underset{\text{Oxalic acid}}{6\,(COOH)_2} + 5\,H_2O$$

23. SYNTHESIS OF ANILINE FROM NITROBENZENE

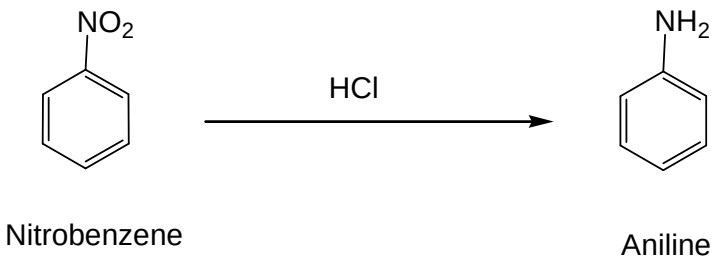
In aromatic electrophilic substitution, in which $–NH_2$ group directs the next incoming group to ortho and para positions in the same molecule. Therefore, amino group is called as ortho & para director, other examples include –OH, -OR, Cl, Br, etc.

Aniline also known as phenyl amine, an aromatic amine, is prepared by reduction of nitrobenzene with as a catalyst in gaseous phase. It is a colourless liquid. its boiling point is 184^oC.it has a paints characteristic odour. It is sparingly soluble in the water but readily soluble in ethanol, ether and chloroform.

PROCEDURE:-

Take 25 gm of tin a round bottomed flask, and 15.5 mL of nitrobenzene is added & fit in the reflux condenser. 10 mL of conc. HCl is then poured & flask is shaken. Then the reaction mixture is refluxed for 1h. Then the flask was reacted on water bath & then solution was made alkaline by adding 50mL NaOH after it was being cooled by adding crushed ice.

CHEMICAL REACTION:-



24. SYNTHESIS OF PHENYTOIN

Phenytoin is an antiepileptic drug. It is synthesized in two steps starting from benzoin. Pinacole-pinacolone mechanism is involved in its synthesis.

PROCEDURE:-

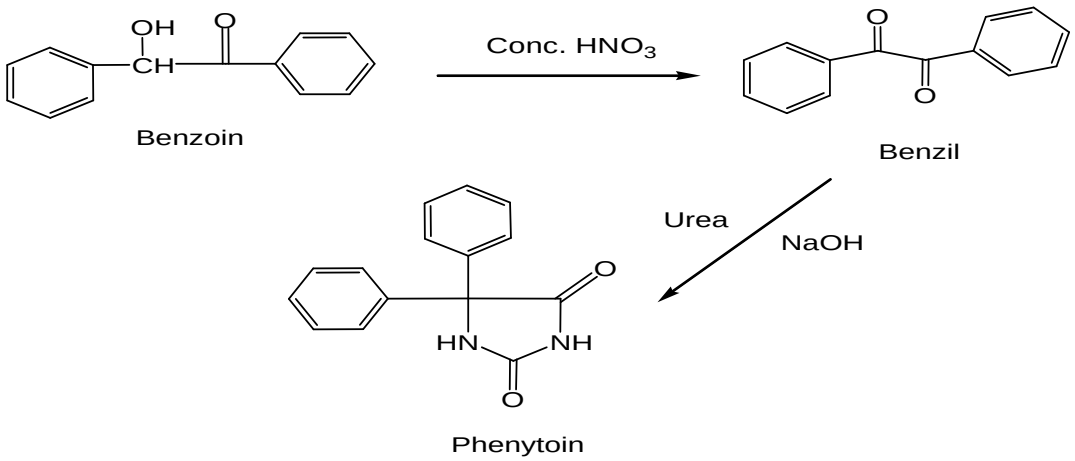
Step-1: Preparation of benzil from benzoin.

Heat a mixture of benzoic (1 gm), glacial acetate acid (15 mL) & conc. HNO_3 (25 mL) in a beaker on boiling water bath for 1 hr then pour down the content into cold water with shaking. Filter out the product, wash & recrystallized with methanol.

Step-2: Preparation of phenytoin from benzil.

Take benzil & urea in 30 % aq. NaOH solution. Add ethanol & heat the content on a water bath for 2 hr & pour down the content into ice cold water. Filter it & add conc. HCl to filtrate. Filter out the product, wash & recrystallize with methanol.

REACTION SCHEME:-



IP STANDARDS:

Phenytoin contains not less than 98.5% and not more than 101.5% and not more than 100.5% of $C_{15}H_{11}N_2O_2$ calculated with references to the anhydrous substances.

IDENTIFICATION:-

Test: A

Dissolve 0.25 gm of drug in 5 mL of water & acidify with dil. HCl. A white ppt is formed which confirms that the drug is phenytoin.

Test: B

Dissolve 0.25 gm of drug in 10 mL of 10% w/v pyridine solution. Add 1 mL of cupric sulphate and allow to stand for 10 min, a blue ppt is formed that confirms that the drug is phenytoin.

Test: C

Melting point of phenytoin is 293 -295 °C.

25. SYNTHESIS OF HYDANTOIN

Hydantoin, or glycolylurea, is a heterocyclic organic compound with the formula $CH_2C(O)NHC(O)NH$. It is a colorless solid that arises from the reaction of glycolic acid and urea. It is an oxidized derivative of imidazolidine. In a more general sense, hydantoins can refer to a groups and a class of compounds with the same ring structure as the parent. Hydantoin is used for the preparation of various important compounds including several antiepileptic drugs

PROCEDURE:-

Step-1: Preparation of hydantoic acid

Aq. NaOH solution is added to a mixture of glycine and urea in a R.B.F. The mixture is shaken well ad heated at 110-115°C for 1 hr. It is cooled to 60°C and then acidified with conc. HCl to congo red after that Filter out the product, wash & recrystallize with methanol.

Step-2: Preparation of hydantoin

A mixture of hydantoin acid (1 gm), conc. HCl and water is heated at 110-115°C for 15 min. A solid product is formed which is separated, filtered and recrystallized from water.

REACTION SCHEME:

Hydantoic acid Hydantoin

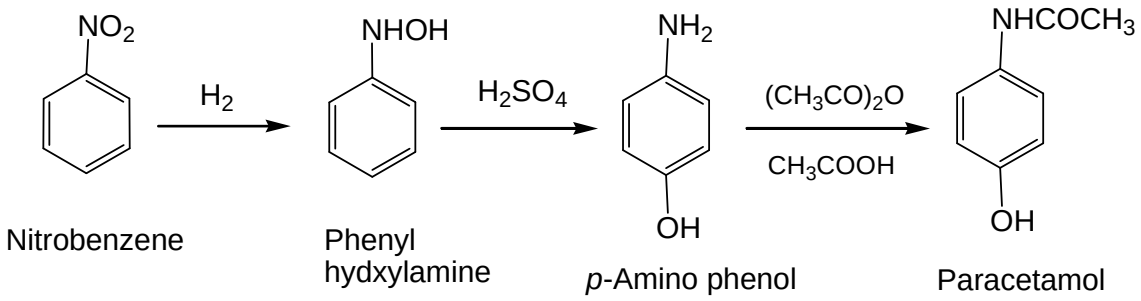
26. SYNTHESIS OF PARACETAMOL

Paracetamol, also known as acetaminophen, is used as antipyretic drug. It is an over the counter drug (OTC).

PROCEDURE:-

1. Suspend 11gm of *p*-aminophenol in 30 mL of water contained in a 250 mL beaker and adds 12 mL of acetic anhydride.
2. Stir the mixture vigorously and warm on a water bath.
3. After 10 min. cool, filter the solid acetyl derivative at the pump and wash with cold water.

REACTION SCHEME:-



IP STANDARDS:

Paracetamol contains not less than 90 % and not more than 101% of $C_8H_9NO_2$ calculated with references to the anhydrous substances.

IDENTIFICATION:

Test: A

Dissolve 50 mg of drug in a sufficient methanol to produce 100 mL. 1 mL of this solution is added to 0.5 mL of 1M HCl & diluted it to 100 mL with methanol then measure the absorbance at the maximum at about 249nm, it is about 0.44.

Test: B

Boil 0.1 gm of HCl acid for 3 min., add 10 mL of water & cool no ppt is produced add 0.05 mL of 0.0167 M $K_2Cr_2O_7$ a violet colour produced which does not turn red.

Test: C

Gives the reactions of acetyl group

27. SYNTHESIS OF BENZOIC ACID

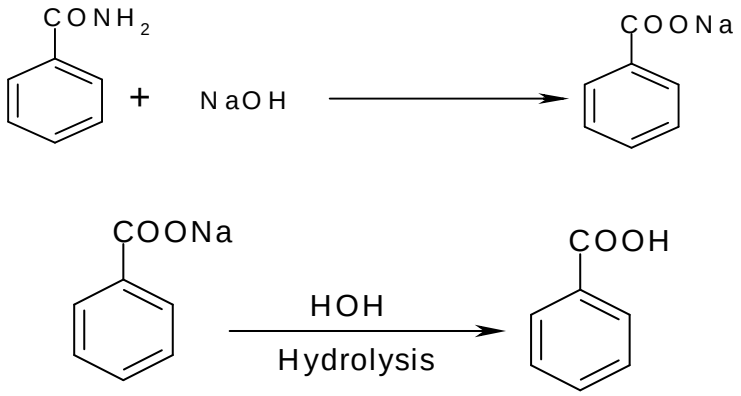
Benzoic acid is simplest aromatic carboxylic acid. It is used for the preparation of various important compounds. Itself it acts as keratolytic and antifungal agent.

PROCEDURE: -

Placed the mixture of conc. NH_3 (10 mL) and water (5 mL) in a conical flask & shake vigorously and add benzoyl chloride drop by drop formed the benzamide as separated.

Take 1 gm of formed benzamide & 10 % NaOH (15 mL) in a RBF flask fitted with refluxes condenses and boil the mixture gently 30 min. ammonia is evolved then add con. H_2SO_4 after cooling the mixture until it becomes acidic ad benzoic acid is immediately separated out.

REACTION SCHEME:



IP STANDARDS:

Benzoic acid contains not less than 99.5 % and not more than 100.5% of $C_7H_6O_2$ calculated with references to the anhydrous substances.

IDENTIFICATION:

Test: A

Warm gently 0.2 gm with 20 mL of water, add 1mL of dil NaOH and filter. To the filtrate add ferric chloride a buff colored is produced.

Test: B

A 1% w/v solution is acidic to methyl red solution.

Test: C

Melting point of benzoic acid is found to be 122 -123º C.

28. SYNTHESIS OF ANTHRANILIC ACID

Anthranilic acid (or *o*-amino-benzoic acid) is an aromatic acid with theformula $C_6H_4(NH_2)(CO_2H)$. The molecule consists of a substituted benzene ring, hence is classed as aromatic, with two adjacent, or "*ortho*-" functional groups, a carboxylic acid and an amine. The compound is consequently amphoteric. In appearance, anthranilic acid is a white solid when pure, although commercial samples may appear yellow. It is sometimes referred to as vitamin L_1 and has a sweetish taste

Anthranilic acid is an important organic compound, and it is used for synthesis of some important drugs. Industrially, anthranilic acid is an intermediate in the production of azo dyes and saccharin.

It and its esters are used in preparing perfumes to imitate jasmine and orange, pharmaceuticals (loop diuretics e.g. furosemide) and UV-absorber as well as corrosion inhibitors for metals and mold inhibitors in soya sauce.

Anthranilic acid can be used in organic synthesis to generate benzyne.

PROCEDURE:-

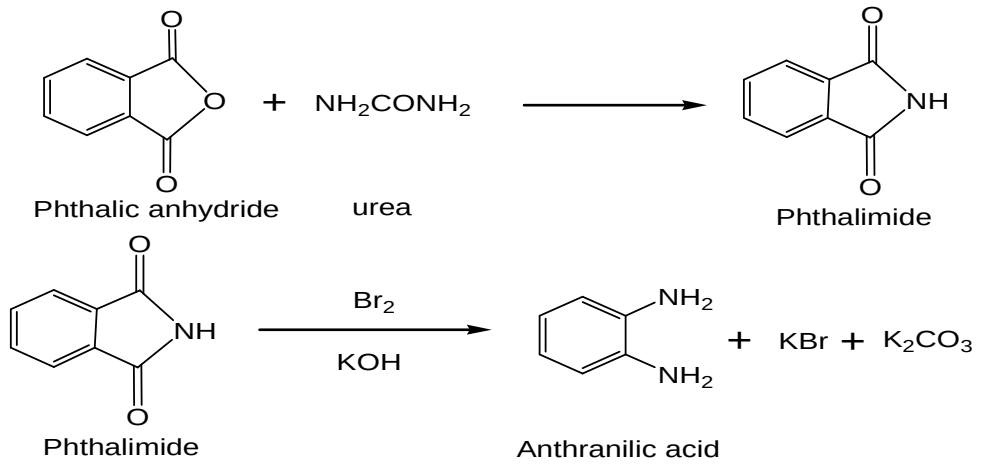
Step-1:

Mix the phthalic anhydride (10 gm) & urea (250 mL) R.B.F. heat the mixture on oil bath till the content melt, froth up & become solid. Remove the flame beneath the bath and allow to cool add water to disintegrate the solid filter the crude product & recrystallized from the ethanol to obtain the product.

Step-2:

Dissolve the NaOH (7.5 gm) in water (40 mL) & cool in ice then add phthalamide and 10% KOH solution, after that heat the solution till phthalimide dissolves. Neutralize the solution with water & recrystallized.

REACTION SCHEME:-



IP STANDARDS:

Anthranilic acid contains not less than 99.5 % and not more than 100.5% of $C_7H_7NO_2$ calculated with references to the anhydrous substances.

IDENTIFICATION:

Test: A

Mix with aniline and dil. Water then added bleaching powder not show the violet coloration.

Test: B

The melting point of the phthalic anhydride was found to be 147-148°C by melting point apparatus.

29. SYNTHESIS OF PICRIC ACID

Picric acid is 2,4,6-trinitro-phenol. It is yellow in colour. It is used as tropical anti infective and disinfectant so that used as a cleaning agent and also preservative due to presence of phenol.

Picric acid is obtained by nitrating phenol. In pharmacology experiments, picric acid is also used to mark the rats and mice.

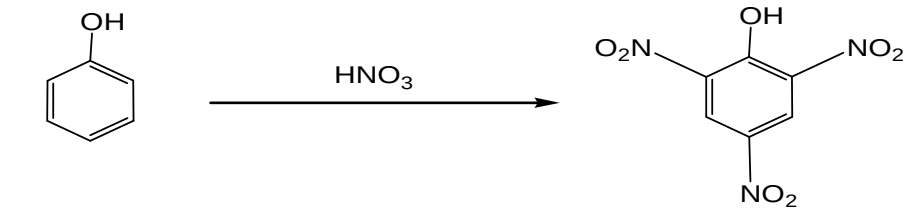
Nitration: Nitration is an example of electrophilic aromatic substitution reaction. A large no. of aromatic compounds can be easily nitrated. The hydrogen atom replaced by nitro group. The nitration of aromatic compounds is usually done by using conc. HNO_3 in presence of conc. H_2SO_4. Nitration of aromatic compounds is an example of electrophillic aromatic substitution. H_2SO_4 not only provides strong acedic medium but it also converts the HNO_3 into reactive electrophile nitronium ion (NO^+_2) which attacks the aromatic ring.

Nitration is usually carried out at low temperature. At high temperature there is loss of material due to oxidation by HNO_3. Phenol being an activated nucleus towards electrophillic aromatic substitution, the nitration reaction occurs very easily. It undergoes nitration with HNO_3 even at room temperature forming ortho & para nitrophenol which can be separated by steam distillation. Phenol when treated with conc. HNO_3 in presence of conc. H_2SO_4 undergoes nitration at both ortho and para position to yield picric acid. It is better if phenol is first converted into phenol sulphonic acid by treatment with H_2SO_4 and then nitrated with conc. HNO_3.

PROCEDURE:

Take 4gm/mL of phenol into a dry 250 mL of conical flak. Add 5 mL of conc. H_2SO_4 & mix thoroughly which becomes warm because the reaction is exothermic, now heat the flask on the boiling water bath for 30 mins to complete the formation of phenol sulphonic acid & then chill the flask thoroughly in ice water mixture. Place the flask on a wooden block, add immediately 15 mL of conc. HNO_3 and at once mix the liquid by shaking for few minutes. Then allow the mixture to stand undisturbed. Usually within 1 min a vigorous but harmLess reaction occurs and red fumes pour out of the flask when the action subsides, heat the flask on boiling water bath for 1-2 hrs with occasional stirring. During this period, the heavy oils which is present at the beginning ultimately forms a mass of crystals. Add 100 mL of cold water and then chill thoroughly, mixing well. Filter the yellow crystals wash thoroughly with water to eliminate all inorganic acids & drain.

CHEMICAL REACTION:-



Nitrobenzene

Picric acid

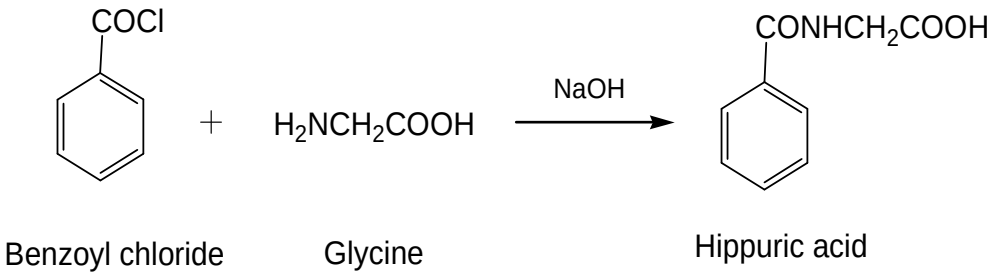
30. SYNTHESIS OF HIPPURIC ACID

Hippuric acid is benzoyl glycine. It is synthesized from the amino acid glycine. Several compounds are synthesized from hippuric acid. It was isolated from horse urine, thats why its name is hippuric acid.

PROCEDURE: -

Glycine (10 gm) is dissolved in 100 mL of 10% NaOH in a conical flask. Then 18 mL of benzoyl chloride is added until all chloride has reacted. The reaction mixture is transferred into a beaker and the conical flask is rinsed with little water. Few grams of ice is placed into the reaction mixture. 1-2 mL of HCl is added & stirred vigorously until reaction mixture gets fully reacted with it. A precipitate is obtained which is filtered, washed thoroughly with water and finally with carbon tetrachloride. It is dried in dry air oven and recrystallized.

CHEMICAL REACTION:-



31. SYNTHESIS OF BARBITURIC ACID

Barbituric acid (malonyl urea or 6-hydroxyuracil) is an organic compound based on a pyrimidine heterocyclic skeleton. It is an odorless powder soluble in water. Barbituric acid is the parent compound of barbiturate drugs, although barbituric acid itself is not pharmacologically active.

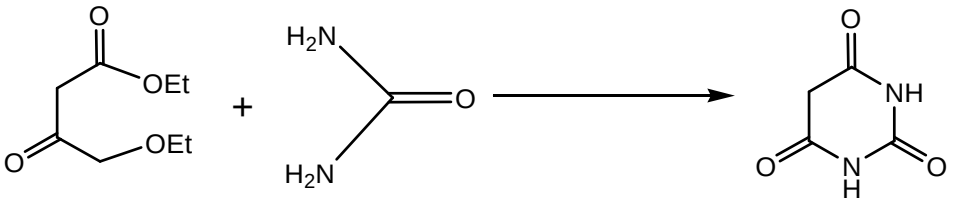
Barbituric acid is used for synthesis of various hypnotic and sedative agents. These derivatives have profound effect on central nervous system.

PROCEDURE: -

In RBF place 11.5 gm sodium & add 250 mL absolute ethanol. When all sodium has reacted add 80 gm of diethyl malonate followed by 30 gm urea solution in 250 mL of hot absolute ethanol. Shake well the mixture fit a calcium guard tube to the top of condenser & refluxes the mixture for 1 hr in the oil bath heated to 110 °C.

A white solid separated treat it with 450 mL hot water & then with conc. HCl continuous stirring until the solution is acidic. Filter the resulting clear solution & keep it in a refrigerator.

CHEMICAL REACTION:



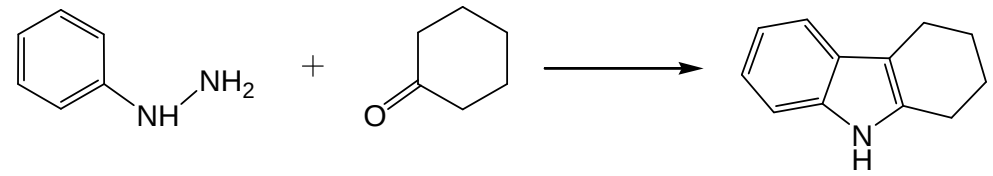
Barbituric acid

32. SYNTHESIS OF 1,2,3,4-TETRAHYDROCARBAZOLE

Tetrahydrocarbazoles can be prepared from cyclohexanone and phenyl hydrazines in a single step. This ring system is also a part of several drugs.

PROCEDURE:-

Place cyclohexanone (4.75 g; 5 mL) and glacial acetic acid (30 mL) in a round bottom flask and add phenylhydrazine (5.4 g; 4.5 mL). Heat the mixture under reflux for 1 hour and then cool. Filter the brown precipitate using water pump. Wash the solid compound from cold water (4x50 mL). Leave the precipitate to dry at room temperature. Crystallize the product from aqueous alcohol.



Phenyl hydrazine Cyclohexanone 1,2,3,4-Tetrahydro Carbazole

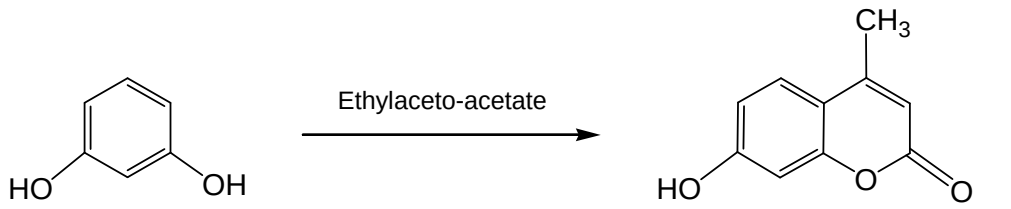
33. SYNTHESIS OF 7-HYDROXY-4-METHYL COUMARIN

The most famous coumarin is warfarin which is used in low doses in humans as a blood thinner. Coumarin can be prepared by the reaction of resorcinol with β- keto ester as ethyl acetoacetate in presence of acid condensation agents (Pechmann reaction).

PROCEDURE:-

Place in 100 mL round bottom flask, resorcinol (5.5 g) and Ethyl acetoacetate (6.5 g) with sulphuric acid (50 mL, 75%). Heat the mixture on water bath at 100 ºC for half hour. Cool the dark green solution with stirring in crushed ice. Filter the precipitate and wash the compound with cold water (100 mL). Crystallize the product from methanol.

CHEMICAL REACTION:-



Resorcinol

7-Hydroxy-4-methyl coumarin

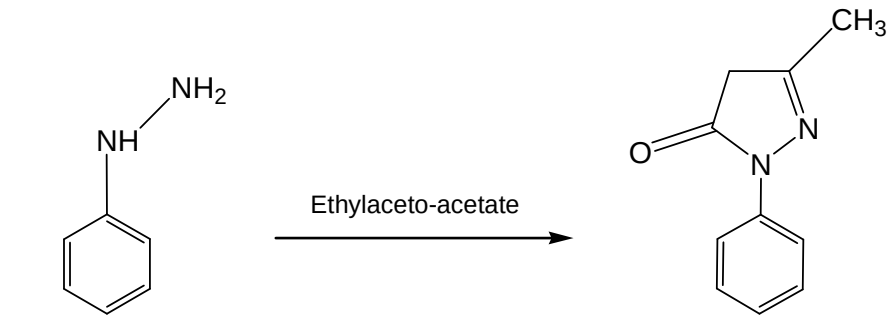
34. SYNTHESIS OF 3-METHYL-1-PHENYL-5-PYRAZOLONE

Condensation of ethyl acetoacetate with phenylhydrazine produces phenylhydrazone derivative which cyclised to pyrazolone derivative. The pyrazole containing compounds show prominent biological actions. They are also used to prepare several pyrazolone-based drugs.

PROCEDURE:-

Place in 100 mL round bottom flask, freshly distilled phenyl hydrazine (5.4 g; 6.35 mL) and Ethyl acetoacetate (6.4 g; 4.9 mL). Heat the mixture under reflux at 120 °C for 1 hour. Cooling the red oily solution and add the ether (50 mL) with stirring to solidification the product. Filter the precipitate. Recrystallize the product from aqueous ethanol (50%).

CHEMICAL REACTION:-



Phenyl hydrazine

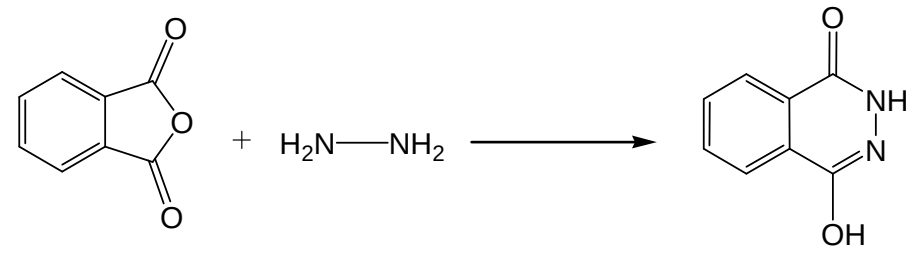
Pyrazolone

35. SYNTHESIS OF 3,4-DIHYDRO-1-HYDROXY-4-OXOPHTHALAZINE

The most famous coumarin is warfarin which is used in low doses in humans as a blood thinner. Coumarin can be prepared by the reaction of resorcinol with β-keto ester as ethyl acetoacetate in presence of acid condensation agents (Pechmann reaction).

PROCEDURE:-

Place in 100 mL round bottom flask, hydrazine hydrate (5.5 g) and phthalic anhydride (3 g) in ethanol (25 mL). Heat the mixture on water bath at 100 ºC for half hour. Cool and filter the solution and then wash by petroleum ether (3 x 40 mL). Reduce the volume of solvent and keep at room temperature, a solid separates.



Phthalic anhydride

Oxophthalazine

36. SYNTHESIS OF IODOFORM

Iodoform is used as a rubifacient. It is a topical analgesic creating heat on applied area. It also acts as antiseptic because of presence of iodine in its structure.

Iodoform is synthesized by oxidation process. Oxidation is the process in which loss of one or more electron, the reagent which undergoes oxidation is called reducing agent. $KMnO_4$ in alkaline medium and $K_2Cr_2O_7$ in acetic medium or even nitric acid is most commonly used reagent for oxidation of organic compounds. Primary alcohols are oxidized to corresponding aldehydes, which on further oxidation gives carboxylic acid group, whereas secondary alcohols are to ketones. Aromatic compounds containing alkyl side chain, but not the group like OH & NH_2 which are affected by oxidizing agents are oxidized to carboxylic acids.

Many organic compounds which contain alcoholic group or ketone group on treatment with KI and NaOCl give Iodoform.

Examples of 1st category compounds are: ethanol, isopropanol or lactic acid [$(CH_3)_2CHOH$].

2nd categories of compounds are: acetone, acetophenones, ($C_6H_5COCH_3$), pyruvic acid $CH_3COCOOH$.

Iodoform can be very easily prepared by acetone by the action of 'NaOCl' in presence of 'KI'

Acetone is 1st converted into triiodo acetone which in presence of alkali is immediately converted into iodoform and sodium acetate.

PROCEDURE:-

Place 0.5 mL of acetone, 20 mL of 10% of KI solution and 8 mL of 10% NaOH solution in 250 mL of conical flask and then add 28 mL of NaOCl solution (5% chlorine). Mix the content well in conical flask. Yellow iodoform begins to separate, allow the mixture to stand at room temperature for 10 minutes and then filter, wash with cold water, & drain thoroughly.

CHEMICAL REACTION:-

$$CH_3COCH_3 + 3KI + 3NaOCl \longrightarrow CH_3COCI_3 + 3KCl + 3NaOH$$

$$CH_3COCI_3 + NaOH \longrightarrow CHI_3 + CH_3CO_2Na$$

37. SOME IMPORTANT/SPECIAL REAGENTS AND THEIR PREPARATION

Alizarin

Make a saturated solution in ethanol.

1-Amino-1-Naphthol-4-sulfonic acid

Dissolve 0.2 g in 195 mL of sodium bisulphite solution (3 in20) and 5 mL of anhydrous sodium sulphite solution (1 in 5) and filter if necessary. Stopper and store in a cool dark place. Use within 10 days.

Ammoniacal silver nitrate

Add conc ammonia to bench silver nitrate (0.1M) until the initially formed ppt. Just disappears.

Ammonium citrate, lead free

Dissolve 40g of citric acid in 100 mL of water and make alkaline to phenol red with ammonium hydroxide. Remove lead by shaking with small portions of dithizone extraction solution in chloroform until the dithizone solution retains its original green color.Discard the extraction solution.

Ammonium molybdate reagent

Method A: Dissolve 45 gms. of the commercial salt or 40 gms of pure molybdenum trioxidein a mixture of 70 mL.of concentrated ammonia solution and 140 mL. of water; when solution is complete, add it very slowly and with vigorous stirring to a mixture of 250mL.of concentrated nitric acid and 500mL.of water, and dilute to 1 litre.Allow to stand 1 to 2 days and decant and use the clear solution.

Method B: Dissolve 45 gms. of pure commercial ammonium molybdate in mixture of 40mL. concentrated ammonia solution and 60 mL. of water, add 120gms. of ammonium nitrate and dilute to a litre with water.

Note: The alkaline solution of ammonium molybdate keeps better than the nitric acid solution; there is little tendency for the separation of solid. Before using the alkaline solution, it is important that the test solution contains a slight excess of nitric acid.

Ammoniacal silver nitrate

Add ammonia dropwise to a 1 in 20 solution of silver nitrate until the ppt. that first forms is almost , but not entirely, dissolved. Filter and store in dark bottle.Forms explosive compounds on standing! Prepare fresh.

Anthranilic acid

Dissolve 0.5g in 100 mL ethanol

Anthrone

Dissolve about 0.1 g in 100 mL sulphuric acid. Prepare fresh.

Aqua Regia

Mix one part by volume of conc. nitric acid with three (3) parts by volume of conc. hydrochloric acid in a pyrex beaker and allow to stand until a bright red color develops.

Barfords reagent

Dissolve 13.3gm.of crystalised neutral copper acetate in 200mL.of 1% acetic acid solution. This reagent does not keep well.

Bradys reagent (2,4 DNPH)

Dissolve 40g of 2, 4 dinitrophenylhydrazine in 80mL conc. Sulphuric acid. Cool and add 900mL methanol and 100mL water.

Benedicts solution (qualitative)

Dissolve 86.5gm.of sodium citrate and 50gm. of anhy. sodium carbonate in about 350mL. of water. Filter if necessary. Add a solution of 8.6gm. of copper sulphate in 50mL. of water with constant stirring. Dilute to 500mL. The resulting solution should be perfectly clear; if not, filter through a fluted filter paper.

Benedicts solution (quatitative)

Dissolve 200g sodium citrate, 75g sodium carbonate and 125g potassium thiocynate in about 600 mL water. Dissolve separately, 18g copper (11) sulphate in 100 mL water. When the solutions have cooled, mix them together with stirring. Now add 5 mL of a 5% potassium ferrocyanide to the solution, and make up to 1 liter.

Benzidine

Dissolve 50 g in 10 mL glacial acetic acid, dilute to 100 mL with water and mix. (caution: Toxic!).

Biuret reagent

Take enough urea to cover the bottom of a test tube. Heat very gently , until the liquid which forms resolidifies. This white solid is biuret. Dissolve the biuret in about 2mL. water, and use this solution for the biuret test.

Or

Soln. A : 0.1m sodium hydroxide. SoLn. B : 0.01M copper (11) sulphate solution. Add soln. A first to sample, then add B. Pink or purple color confirms protein.

Carr-Price reagent

Weigh an unopened bottle of antimony trichloride. Open the bottle and empty the contents into a wide mouth glassstoppered amber bottle containing about 100 mL of chloroform. By difference, obtain the weight of antimony trichloride and then add sufficient chloroform to supply 100 mL for each 25 g. Dissolve by warming or shaking for several hours and filter through sodium sulphate into a dry clean amber bottle with ground stopper.Store at room temperature and keep in dark when not in use. Rinse all glassware coming into contact with this reagent wit chloroform or a mixture of ethanol and ether, since the antimony oxychloride which forms is insoluble in water.

Chromic acid

Weigh out 10 g of sodium dichromate crystals, make it into a slurry with a few mLs of watwer, then dissolve in 250mLs conc. sulphuric acid with stirring and cooling (ice-bath), to give a thick syrupy dark brown mixture.(very corrosive!)

Deniges reagent

Dissolve 5 g of yellow mercuric oxide (HgO) in a mixture of 40 mL of water,and while stirring slowly add 20 mL of sulphuric acid, then add another 40 mL of water, and stir until complety dissolved <0.5N)

Dimethyl glyoxime

Dissolve 1gm. of the solid in 100mL. of 95% ethyl alcohol.

N,N-dimethyl-p-phenylenediamine

Measure and pour into a 250 mL beaker 89 mL of distilled water. Stir on a magnetic stirrer. Carefully add 15 mL of concentrated sulfuric acid. Add and dissolve 1.0 g n,n-dimethyl-p-phenylenediamine sulfate. Add 5 g of Florisil and stir the mixture until all is absorbed. Allow the adsorbant to settle and decant the supernatant solution.

Diphenylcarbazide

Use a saturated ethanolic solution; or dissolve 0.125 g in a mixture of 25mL acetone 25 mL water; or dissolve 0.2g in 10 mL acetic acid and dilute to 100 mL with methanol

Diphenylcarbazone

Dissolve an approximately 1% solution in ethanol.

Dithiol reagent

4-Methyl-1:2 dimercarpo-benzene

Dissolve 0.2g in 100 mL of 1% NaOH

Dithizone solution

Dissolve 30 mg (milligram) of dithizone in 1 liter chloroform, add 5 mL alcohol, and store in refrigerator.

Dragendorff reagent

Solution 1: dissolve 0.85g of basic bismuth nitrate in 10 mL acetic acid and 40 mL water.

Solution 2: dissolve 8 g of potassium iodide in 20 mL water.

Mix 5 mL of Solution 1; 5 mL of Solution 2; 20 mL of acetic acid; and 100 mL of water before use.

Ethylenediamine reagent

Add copper sulphate to a solution of ethylenediamine until the color becomes dark-blue violet

Fehlings solution

Solution A; Dissolve 34.6 gms of pure copper sulphate in distilled water and dilute to 500mL. (blue colour).

Solution B: Dissolve 173gms of sodium potassium tartrate and 30gms of pure sodium hydroxide in water and dilute to 500mL. Alternately, dissolve 121gms of pure sodium hydroxide and 93.1gms of pure tartaric acid in water, then dilute the solution to 500mL (colourless). Mix equal volumes of solutions A and B immediately before use, and then use as the reagent.

Ferric thiocyanate reagent

Dissolve 1.5 g of ferric chloride and 2.0 g of potassium thiocyanate in 100 mL water.

Ferron reagent

7-iodo-8-hydroxyquinoline-5-sulphonic acid

Dissolve 0.2 g in 100 mL water

Fluorescein

Make a saturated solution in 50% ethanol.

Formaldehyde

Dilute the commercial 40% solution (1 part) with water (7 parts)

Fuchsin solution

Dissolve 0.15gm. of fuchsin in 100mL. water.

Hydrazine sulphate

Dissolve 2 g in 100 mL water.

Hydrogen peroxide

Use the commercial 10 volume (3%) or 20 volume 6%) solution.

Hydroxylamine hydrochloride

Dissolve 10 g in 100 mL water.

8-Hydroxyquinoline

Use a 5% solution in ethanol.

Indigo solution

Gently warm 1gm. of indigo with 12mL. of concentrated sulphuric acid, allow to stand for 48hrs. and pour into 240mL. of water. Filter if necessary.

Indole

Dissolve 0.15 g in 100 mL ethanol

Iodine reagent

Dissolve 20g KI and 10g iodine crystals in 100mL water.

Jones reagent

Mix 25g of chromium trioxide (chromic anhydride CrO3) with conc. sulphuric acid to a paste, then dilute with water to 75mLs.

Karl Fisher Reagent

Dissolve 762 g of iodine in 2,420 mL of pyridine in a 10 liter glass stoppered bottle, and add 6 liters of methanol. To prepare the active stock, add 3 liter of the foregoing stock to a 4 liter bottle, cool in ice bath. Add carefully 135 mL of liquid sulfur dioxide, collected in a calibrated cold trap, and stopper the bottle. Shake the mixture until homogeneous, and set aside for one or two days before use.

Magnesia mixture

Dissolve 100gms of magnesium chloride and 100gms of ammonium chloride in water, add 50mL. of concentrated ammonia solution and dilute to 1 litre with distilled water.

Magnesium nitrate reagent

Dissolve 130gms of magnesium nitrate and 200gms of ammonium nitrate in water, add 15-20mLs.concentrated ammonia solution and dilute to 1 litre.

Malachite green

A 1% solution of malachite green oxalate in glacial acetic acid.

Manganese sulphate

Dissolve 90 gms manganese sulphate in 200 mL water, 175 mL phosphoric acid and 350 mL of diluted sulphuric acid (1 in2). Add water to make up 1 liter.

Mayers reagent

Mercuric-Potassium Iodide

Dissolve 1.358 gms of mercuric chloride in 60 mL water. Dissolve 5 gms of potassium iodide in 10 mL water. Mix the two solutions and add water to make 100 mL.

Millons reagent

Warm one globule of Mercury with concentrated nitric acid and dilute the solution with twice its volume of water.

Molischs reagent.

20% soln. in naphthol. Dissolve 20 gm of 1-naphthol in 100 mL ethanol.

Murexide

Add 0.4 gm of murexide to 40 gm of powdered potassium sulphate, and grind in glass mortar to a homogeneous mixture.

Naphthalenediol

Dissolve 0.1 gm of 2,7-dihydroxynaphthalene in 1 liter sulphuric acid and allow the solution to stand in the dark until the yellow color has disappeared (at least 18 hr).

1-Naphthol

Dissolve 1 gm of 1-naphthol in 25 mL methanol. Prepare fresh.

Nessler reagent

Dissolve 10 gm of potassium iodide in 10 mL of ammonia-free water, adding saturated mercuric chloride solution (60 gm/litre) in small quantities at a time with shaking, until a slight permanent precipitate is formed, then adding 80 mL of 9M sodium hydroxide solution and diluting to 200 mL. Allow to stand overnight and decant the clear liquid.

Nessler reagent has been described as a solution which is about 0.09M in potassium mercuri-iodide and 2.5M in potassium hydroxide.

An alternative method is to dissolve 23 gm of mercuric iodide and 16 gm of potassium iodide in ammonia-free water and make up the volume to 100 mL; add 100 mL of 6M sodium hydroxide. Allow to stand for 24 hr and decant the solution from any precipitate that may have formed, the solution should be kept in the dark.

Another method that reacts promptly and consistently is to dissolve 143 gm of sodium hydroxide in 700 mL water. Dissolve 50 gm of red mercuric iodide and 40 g of potassium iodide in 200 mL water. Pour the iodide solution into the hydroxide solution, and dilute with water to 1 liter. Allow to settle, and use the supernatant liquid.

Ninhydrin

A 0.2% solution of ninhydrin (triketohydrindene hydrate, $C_9H_4O_3.H_2O$) in water. Prepare fresh.

1,10-phenanthroline

Dissolve 0.1 gm of the monohydrate in 100 mL water.

Orthophenanthroline

Dissolve 0.15 gm orthophenanthroline ($C_{12}H_8N_2.H_2O$) in 10 mL solution of ferrous sulphate, prepared by dissolving 1.48 gm of ferrous sulphate in 100 mL water. The ferrous sulphate solution must be prepared immediately before dissolving the orthophenanthroline.

Picric acid

Dissolve the equivalent of 1 gm of anhydrous 3,4,5-trinitro-phenol in 100 mL of hot water. Cool, and filter if necessary.

Quimociac reagent

Dissolve 70 gm of sodium molybdate ($Na_2MoO_42H_2O$) in 150 mL water (Solution A). Dissolve 60 gm of acetic acid in a mixture of 85 mL nitric acid and 150 mL of water and cool (Solution B) Gradually add Solution A to Solution B, with stirring, to produce Solution C. Dissolve 5 gm of synthetic quinoline in a mixture of 35 mL nitric acid and 100 mL water (Solution D) Gradually add Solution D to Solution C, mix well, and allow to stand overnight. Filter the mixture, add 280 mL of acetone to the filtrate, dilute to 1 liter with water, and mix. Store in a polythene bottle. (Caution: Flammable).

Quinaldic acid

Neutralize 1gm of the acid with NaOH and dilute to 100 mL

Quinalizarin reagent

Dissolve 0.02gm in 100 mL ethanol, or dissolve 0.05gm in 100 mL of 0.01M sodium hydroxide.

Rhodamine B

Dissolve 0.01 gm in 100 mL water, or dissolve 0.05 gm rhodamine B and 15gm KCL in a solution of 15 mL conc. HCl and 85 mL water.

Salicylaldehyde

A 20% solution in ethanol.

Schiffs reagent

Method 1: Dissolve 0.2 gm of pure *p*-rosaniline hydrochloride in 20 mL of a cold, freshly prepared, saturated aqueous solution of sulphur dioxide; allow the solution to stand for a few hours until it becomes colourless or pale yellow. Dilute the solution to 200 mL and keep it in a tightly stoppered bottle. The solution keeps well, and should not be exposed to light or air. Store in the dark.

Method 2: Add 2 gm of sodium bisulphite to a solution of 0.2 gm of *p*-rosaniline hydrochloride and 2 mL of concentrated hydrochloric acid in 200 mL of water.

Silver ammonium nitrate

Dissolve 1 gm of silver nitrate in 20 mL of water. Add ammonia dropwise, with constant stirring, until the ppt. is almost but not entirely dissolved. Filter and store in dark container.

Silver diethyldithiocarbamate

Dissolve 1 gm in 200 mL of freshly distilled pyridine.

Sodium borohydride

Dissolve 0.6 gm sodium borohydride and 0.5gm of sodium hydroxide with stirring and dilute to 100 mL with water.

Sodium ethoxide

Dissolve 10 gm of sodium metal in 120 mL of ethanol using the following method: remove surplus oil from sodium with filter paper, dry again on filter paper, and cut the weighed metal into small pieces about the size of a pea. Pour the ethanol into a 500 mL flask cooled on ice bath, and add one or two pieces at a time until dissolved.

Sodium hypochlorite solution

The commercial product contains about 10-14 % w /v of available chlorine. Dilute with an equal volume of water.

Sodium nitroprusside solution

Prepare a solution as required by dissolving a crystal in 5 mL of water.

Starch solution

Triturate 0.5 gm of soluble starch with a little cold water into a thin paste and add 25mL of boiling water. Boil until a clear solution is obtained (5 min). This solution should be freshly prepared as required. Amore stable starch solution is obtained by adding 0.5 gm of potassium iodide and 2-3 drops of chloroform.

A more satisfactory starch solution for use as an indicator is prepared as follows: Mix 5.0 gm of powdered sodium starch glycollate with 1-2 mL ethyl alcohol, add 100 mL of cold water and boil for a few minutes with stirring. This 5% stock solution is stable for many months; it is diluted to 0.1% strength when required for use.

Sulphanilic acid

Dissolve 1 gm in 100 mL of warm 30% acetic acid.

Tannic acid

Dissolve 1 gm of tannic acid (tannin) in 1 mL ethanol, and add water to make 10 mL. Prepare fresh.

Tartrate solution, alkaline

Dissolve 34.6 gm of sodium potassium tartrate (rochelle salt) and 10 gm of sodium hydroxide in water, dilute to 100 mL, let stand for two days, and filter through glass wool.

Thiourea

Dissolve 10 gm of thiourea in 100mL of water (10%).

Titan yellow

Titan yellow is also called thiazole yellow or clayton yellow. Dissolve 5 gm in water, filter and dilute to 100 mL.

Titanium tetrachloride

Cool separately in small beakers surrounded by crushed ice 10 mL of 20% hydrochloric acid and 10 mL of clear, colourless titanium tetrachloride. Add the tetrachloride dropwise to the chilled acid. Stand at ice temperature until all the solid dissolves, then dilute to 1 liter with 20% hydrochloric acid.

Tollens reagent

Add sodium hydroxide soln. to silver nitrate soln. to form a ppt. then add dilute ammonia soln. until ppt. dissolves.

Triton X-100

20% solution: dissolve 0.20 gm of Triton-X-100 (polyethelene glycol ether of isooctylphenol) in water, and dilute to 100mL.

Xylenol orange

Make up a 1% solution in ethanol

Zinc amalgam

Add about 10 gm of granulated zinc to 20 mL mercury, to produce a liquid amalgam on cooling, and heat to 150 ºC with stirringuntil the zinc is dissolved.

Zinc amalgated (Jones Reductor)

The zinc is amalgated by immersing it in a solution of mercuric chloride in hydrochloric acid. A quantity of 250 gm of 20 mesh zinc is covered with water in a 1 liter flask, and a solution of 11 gm of mercuric chloride in 100 mL of hydrochloric acid is poured into the flask. The system is slowly mixed and shaken for about 2 min. The solution is poured off, and the amalgam is washed thoroughly with hot tap water, then distilled water.

Zwikkers reagent

Mix 1 mL of pyridine with 4 mL of a 10% aqueous solution of copper sulphate and 5 mL of water.

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