1

Laboratory Practices

The synthesis and analysis of compounds (inorganic and organic) is an important element of the pharmaceutical courses. The medicinal-organic chemistry laboratory provides a good platform for gaining knowledge about different chemical reactions. The representative reactions and analysis procedure provides adequate knowledge and practice. In general, chemical synthesis and analysis are conducted by dissolving the reactants and analytes in a suitable solvent. The temperature and pressure maintenance for the chemical reactions are important factors. The sequence of addition and speed in which the chemicals and reagents are added are major deciding factor. These factors decide the accuracy of the conclusions, both in synthesis and chemical analysis.

The organic reactions can be conveniently grouped into

1. Microscale reactions
2. Semi-microscale reactions
3. Macroscale reactions

It is very essential to know about the different components of synthesis and chemical analysis. Following are the important components.

1. Knowledge about chemicals
2. Solvents
3. Glassware and equipments
4. Glassware cleaning
5. Purification
6. Drying
7. Determination of melting point and boiling point
8. Yield calculation
9. First aid

1.1 Knowledge about Chemicals

***Class 1* Explosives:** The chemicals may explode under the stimulus of heat, impact or friction. The examples include peroxide, acetylide, azide, diazonium, nitroso, nitro, perchlorate and ozonide compounds. Autoxidation of ethers, tetrahydrofuran (THF) and related compounds forms explosive peroxides. Chromic acid and perchloric acid are known violent explosives.

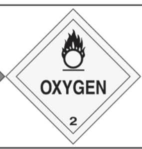
Label	*Background colour*	*Symbol*
  	Orange	Exploding ball

***Class 2* Gases - inhalation:** Gases that are highly flammable, non-flammable and poisonous are included in this class.

Flammable: Hydrogen, butane, methane, ethylene and chlorine trifluoride

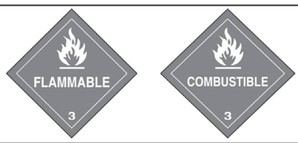
Non-flammable: Nitrogen, carbon dioxide, compressed air and helium.

Poisonous: Arsine, chlorine, phosphine, nitrogen dioxide, hydrogen cyanide and hydrogen azide.

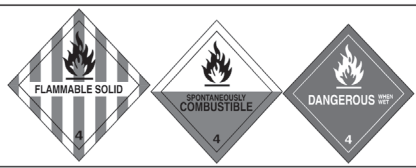
Type	*Label*	*Background color*	*Symbol*
Flammable gases	 	Red or Yellow	Flame
Non-flammable gases		Green	Tube
Poisonous gases		White	Skull and crossbones

***Class 3* Flammable and Combustible Liquids:** Flammable substances are the most common hazard. Flammability of a compound is indicated by flash point. A liquid with flash point less than 15 ºC, is dangerously flammable. The vapour of flammable may drift into a distant ignition and burn back to ignite the bulk source. Organic solvents such as acetone, diethyl ether, ethyl acetate are examples. The flammable liquids should be stored in storage rooms designed.

- ***Flammables:*** Low boiling point compounds such as acetaldehyde, acetone, benzene, carbon disulphide, diethyl ether and ethyl acetate are highly inflammable.
- ***Combustible Liquids:*** Kerosene, diesel oil, engine oil, ethylene glycol and acetic acid are examples of combustible liquid category.

Label	*Background colour*	*Symbol*
	Red	Flame

***Class 4* Flammable Solids:** Flammable solids and spontaneously combustibles are dangerous when they are wet. Examples include metallic hydrides, metallic sodium and potassium, processed metals, nitrocellulose, oily fabrics, etc.

Label	*Background colour*	*Symbol*
	Red and White candy striped Blue if reacts with water	Flame

***Class 5* Oxidizers and Organic Peroxides:** Oxidizers are incompatible with organics, hence oxidizers should be stored separately from organics. Oxidizers promote combustion and produce fire (explosion). Never open an oxidizer container (bottle) with solid formation around the lid. Friction caused by unscrewing the cap leads to an explosion. Organic peroxides, such as Methyl ethyl ketone peroxide (MEKP) and benzoyl peroxide on decomposition produce free radicals and thus useful as initiators of a few polymerization reactions of unsaturated compounds.

Label	*Background colour*	*Symbol*
	Yellow	Flaming ball

***Class 6* Poisons and Etiologic Materials:** The chemicals which are classified as acute toxicity category 1, 2 and 3 under Globally harmonized system (GHS) of Classification and Labelling belong to class 6. Examples are cyanides, lead compounds, phenol, cresol, a few pesticides, clinical wastes and biological wastes.

Label	*Background colour*	*Symbol*
	White with black letters	Varies

***Class 7* Radioactive Materials:** Material containing unstable atoms that emit ionizing radiation as they decay are known as radioactive materials. Enriched uranium, radioactive ores, isotopes (Cobalt 60, iodine 131, iridium 131 and hydrogen 3) and some medical equipments are few examples.

Label	*Background colour*	*Symbol*
	White on the bottom, yellow on the top	Trefoil

***Class 8* Corrosives:** Materials that are highly reactive and cause damage to living tissue are known as corrosives. Acids and bases are common corrosives. Hydrochloric acid, sulphuric acid and sodium hydroxide and batteries are few examples.

Label	*Background colour*	*Symbol*
	Black on the bottom and white on the top	Piece of metal and a hand damaged by liquid

***Class 9* Miscellaneous:** Hazardous materials which not specified in any other classes are included in this class. Marine pollutants, like zinc oxide, lithium ion batteries, genetically modified organisms, air bag modules and motor engines.

Label	*Background colour*
	Seven black vertical strips in upper half with white background, figure 9 is underlined.

1.2 Solvents

A solvent (*In Latin*: loosen, untie) is a substance that dissolves a solute and yields a solution. The quantity of solute that can dissolve in a specific volume of solvent varies with temperature. It also depends on rate of interaction between solute and the solvent.

Classification of solvents:

Three broad categories of solvents are known:

1. Protic/aprotic solvents
2. Polar/apolar (non-polar) solvents
3. Donor/non-donor solvents

A protic solvent consists of molecules that can act as hydrogen-bond donors.

e.g., Water, alcohols and carboxylic acids.

Solvents that cannot act as hydrogen-bond donors are termed aprotic solvents.

e.g., Ether, methylene chloride and hexane.

Polar solvents have high dielectric constant and they contain bonds between atoms with different electronegativities, such as oxygen, nitrogen, halogens and hydrogen. Apolar solvent has a low dielectric constant. They contain bonds between atoms with similar electronegativities, such as carbon and hydrogen.

Borderline polar aprotic solvents: These solvents have moderately higher dielectric constants than the nonpolar solvents. Since they have intermediate polarity they are good "general purpose" solvents for a wide range of reactions. They are "aprotic" because they lack -OH or -NH bonds. They generally serve as the medium.

Polar aprotic solvents

These solvents have large dielectric constants (>20) and large dipole moments, but they do not participate in hydrogen bonding (no -OH or -NH bonds). Their high polarity allows them to dissolve charged species such as various anions used as nucleophiles (e.g., $-CN^-$, $-OH^-$, etc.). The lack of hydrogen bonding in the solvent means that these nucleophiles are relatively "free" in solution, making them more reactive. These solvents do not participate in the reactions.

Polar protic solvents

Polar protic solvents tend to have high dielectric constants and high dipole moments. Furthermore, these solvents participate in hydrogen bonding. These solvents can also serve as acids (sources of protons) and weak nucleophiles (forming bonds with strong electrophiles). They are most commonly used as the solvent for their conjugate bases.

- Donor solvents consist of molecules that can donate unshared electron pairs. These molecules can act as Lewis bases.
  e.g., Ether, tetrahydrofuran (THF), methanol.
- Non-donor solvents cannot act as Lewis bases.
  e.g., Pentane, benzene.

Table 1 Properties of some common organic solvents.

Name	Dielectric constant	Dipole moment (μ)
Non-polar solvents		
Pentane	1.8	0.00
Hexane	1.9	0.00
Cyclohexane	2.0	0.00
Benzene	2.4	0.00
Toluene	2.3	0.36
Chloroform	4.8	1.04
Diethyl ether	4.3	1.15
Borderline polar aprotic solvents		
Dichloromethane	9.1	1.60
Tetrahydrofuran (THF)	7.5	1.75
Ethyl acetate	6.0	1.78

Table *Contd...*

Polar aprotic solvents		
Acetone	21	2.88
Dimethylformamide (DMF)	38	3.82
Acetonitrile	37	3.92
Dimethyl sulfoxide (DMSO)	47	3.96
Polar protic solvents		
Ammonia	16.61	1.4
t-Butanol	12	1.7
n-Propanol	20	1.68
Ethanol	25	1.69
Methanol	33	1.70
Acetic acid	6.2	1.74
Water	80	1.85

For the solubility of covalent compounds, *like dissolves the like* is the thumb of rule. Apolar aprotic solvent is good solvent for another apolar aprotic substance. Protic solvent in which significant hydrogen-bonding interactions occur between molecules is likely to dissolve another protic substance.

Among *n*-butane, ethyl chloride, ethyl methyl ether and 1-propanol, the later one is most soluble. A solution is obtained when the alcohol is mixed with water, as it is a protic. The ability to form hydrogen bonds with water is an important factor in water solubility. Ether contains oxygen that can accept hydrogen bonds from water, but it's not a hydrogen-bond donor. Hence it has some water-like characteristics, but less than the alcohol. In case of alkane and alkyl halide, they can neither donate nor accept hydrogen bonds. Therefore they are least soluble in water.

Among the homologous series of alcohols, higher alcohols (with long hydrocarbon chains) are relatively insoluble in water and more soluble in apolar aprotic solvents. Solvents consisting of polar molecules lie between the extremes of water on one hand and hydrocarbons on the other. As it can accept hydrogen bonds, it dissolves water and alcohols. As its dipole moment can interact favourably with other dipoles, it also dissolves polar compounds like alkyl halides. Due to the presence of its hydrocarbon portion, it can take part in van der Waals interactions, hence dissolves hydrocarbons also.

Ionic compounds in solution can exist in several forms, namely *ion pairs* and *dissociated ions*. In an ion pair, each ion is closely associated with an ion of opposite charge. While dissociated ions move more or less independently in solution and are surrounded by several solvent molecules, called collectively the *solvation shell* or *solvent cage* of the ion. Solvation refers to the favourable interaction of a dissolved molecule with solvent. When solvent molecules interact favourably with an ion, they are said to *solvate* the ion.

Applications

Solvent is a liquid used to dissolve a compound. Solvents have many practical applications:

1. For solubilisation of organic compounds
2. As heat transfer medium
3. Recrystallization

1.3 Glassware and Equipments

In general glassware (reaction vessels) are useful in the following purposes:

- for mixing reactants
- for heating/ boiling reaction mixture and solutions
- for distilling the reaction mixture and extracts
- for cooling the hot solutions
- for collecting the products
- for storing the solutions, reagents and isolated products

Flasks

Reaction flasks: The flasks are designed to tolerate the large pressure differences due to vacuum and pressure (applied and generated).

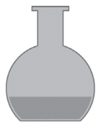
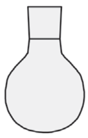
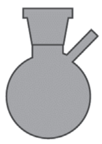
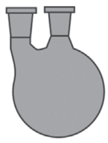
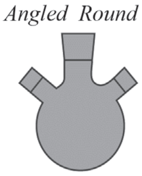
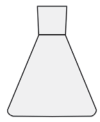
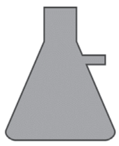
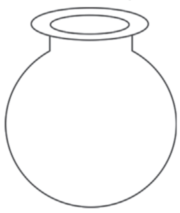
Flat Bottom Flask 	It is also known as Florence flask. This flask is useful for refluxing/ heating the reaction mixture with stirring.
Round bottom flask (bolt-headed flask) 	This flask is useful for refluxing, heating and distillation procedures. The necks of the flasks are connected to the condenser to prevent the solvent evaporation.
Round Bottom Flask with septum inlet 	*Two-Neck Angled Round Bottom Flask* 
Two-Neck Vertical Round Bottom Flask 	*Three-Neck Angled Round Bottom Flask* 

Table ***Contd...***

Erlenmeyer flask (conical flask) 	Cone shaped and flat bottomed cylindrical flasks useful in mixing heating, titration and cooling the reaction mixtures.
Volumetric flask 	This flask is useful in preparing the liquids with high precision volumes (essential for analysis).
Evaporatory flask 	Centered and pear shaped flasks with the socket are useful in solvent recovery (evaporation) procedure in rotary evaporator.
Filtering flask 	It is used along with Buchner's funnel. It has a port for connecting to vacuum. This facilitates the filtration under vacuum (enhanced filtration).
Wide necked flask 	This flask is useful in conducting semi-micro and macro scale experiments.
Iodine flask 	The loss of iodine and other volatile substances (analytes) during the analysis through evaporation is important concern. To overcome this issue Iodine flasks are designed. Iodine flasks differ from Erlenmeyer (conical) flasks, and improve the accuracy of analysis. The difference are: • Ground glass joint: It prevents the evaporation of volatile compounds. • Modified stopper: It provides tight closure. • Funnel shaped top: It prevents the spillage.

Condensers

Condensers are useful in warming, heating, refluxing and distillation process. In heating and reflux process, it condenses the solvent vapours and sends them back to flask thereby reduces the solvent loss. In distillation procedures condenser streamlines the flow of solvent vapour from the flask to the receiver. It provides better separation between the mixture of components.

Types of condensers

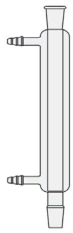
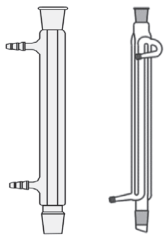
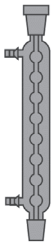
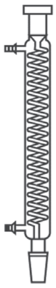
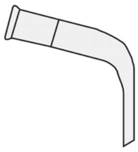
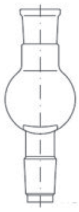
Liebig condenser (west condenser) 	– An inner glass tube surrounded by a glass jacket permits the water circulation. – It allows rapid heat transfer and rapid condensation.
Double surface condenser 	– It contains an inner and outer water jacket for cooling. It is useful for liquids having boiling point below $40\ ^{\circ}C$. – It condenses the liquid vapour between the two jackets (efficient). – Davies type and Double coil type are the two types of double surface condenser known.
Air condenser 	– A glass tube as the inner part of a Liebig-type condenser, useful for liquids with boiling point above $90\ ^{o}C$. – The heat of the liquid will be conducted to the glass, which is cooled by air.
Coil condenser 	An open tube sealed by glass coil/spiral (water circulates in it).

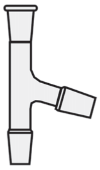
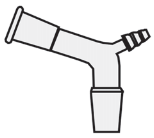
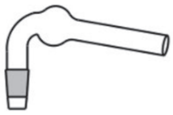
Table *Contd...*

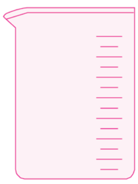
Allihn condenser 	– Long glass tube with water jacket and series of bulbs. – The bulbs on the tube increase the surface area for the vapour condensation. – This setup offers effective means of separation.
Graham condenser (Inland revenue condenser) 	It contains a coolant spiral coil jacket (condensate path) for the circulation followed by vapour condensation.

Distillation Heads

Knee tube 	Mostly utilised in the removal of solvents during distillation.
Claisen distillation head 	The left-hand socket accommodates the capillary tube for use in distillation under vacuum. The right-hand socket is for placing suitable thermometer.
Splash-Guard 	Employed in rotary evaporators and can be used as splash protection (bubble inhibitor).

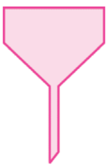
Receiver, Adapters/Connectors

Claisen adapter 	It is used when the reaction requires both reflux and addition of reactants at the same time. One joint connects to condenser for reflux and the other gets connected with dropping funnel (addition of reactants).
Claisen distillation adapter 	This adapter has two connections, one is socket for thermometer and the other one connects to flask.
Vacuum distillation adapter 	This adapter provides connection to vacuum pump to facilitate the distillation under reduced pressure (more suitable for thermolabile substances).
Calcium guard tube (drying tube) 	It is used to exclude moisture from experimental setup during reflux/ distillation. A tube like structure with one end bend, which connects the tube to reaction vessel. The end contains bulb to pack solid desiccant materials (granular alumina, silica gel, calcium sulphite and calcium chloride). A cotton plug (glass wool plug) to the bottom opening prevents the contact of these desiccants to the reaction vessel.



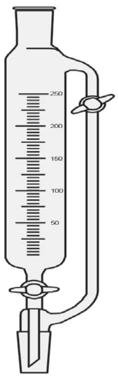
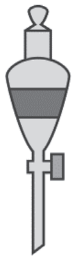
Beakers

Beakers are commonly made up of borosilicate glass, metal (aluminium and stainless steel) but plastic [polythene, polypropylene, polystyrene, polytetrafluoroethylene (PTFE)] beakers are also available for gamma spectral analysis. They are generally graduated, cylindrical with flat bottom and has a spout (beak), which assists in pouring. Griffin with spout and conical beaker are the two common types of beakers in use for stirring, mixing and heating of liquids.

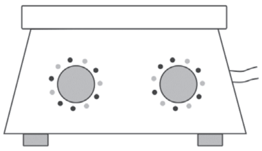


Funnels

Funnels are made up of glass, porcelain or plastics and are useful for the filtration. The hot solution must be rapidly filtered before undue cooling has occurred using fluted filter paper.

Dropping funnel 	It is useful in transferring/adding the liquids and reagents in a controlled manner (dropwise). Because quick addition of liquids and reagents may result in vigorous side reactions.
Separating funnel 	It is useful in separation of mixture of components by distributing (partition) them into two immiscible solvents (liquid-liquid extraction) with different densities.
Buchner funnel 	It is useful in filtering the mixture/suspension to collect recrystallized compounds and generally made up of porcelain. Pouring the suspension to be filtered onto the filter paper placed on the surface of perforated plate of funnel and applying the vacuum through the side arm results in effective filtration. If the same funnel contains smaller plate then it is called as Hirsch funnel. Usually the filter paper is moistened with solvent to prevent the leakage. It provides several fold efficient filtration than normal filtration.
Measuring cylinder 	It is also known as graduating/mixing cylinder. A narrow cylindrical shaped glassware used for the purpose of measuring the liquids such as solvents. It provides more precise measurement than beakers and flasks. But, not preferable for volumetric analysis.

Magnetic Stirrer

Magnetic stirrer 	It is a laboratory device that employs rotating magnetic field to cause a stirrer bar immersed in a liquid to spin very quickly. It ensures the uniform and optimum stirring.
Stirring Rod (Stir Bar/Flea) 	It is a piece of solid glass - magnetic bar generally made up of borosilicate (Pyrex), used for the purpose of mixing chemicals and liquids. The stir bar motion is driven by electromagnets present in the stirrer. They will be coated with Teflon or glass. Glass coating is suitable for the mixing of liquid alkali metals solutions.
Boiling Chips 	Super heating is a condition in which heat will be trapped around the gas surface and there is no release of energy. This is responsible for the formation of bubbles, which converts into gas phase and can be explosive. Super heated solution results in bumping and causes loss of liquid. Stirring and agitation ensures that the heat is properly distributed in the reaction mixture. Use of porous chips, sticks and wooden splints prevents solvent overheating. Air trapped within the porous chip/ stick/wooden splint initiates the bubble formation to the top of the liquid and allows solution to boil evenly.

Heating Reaction Mixtures

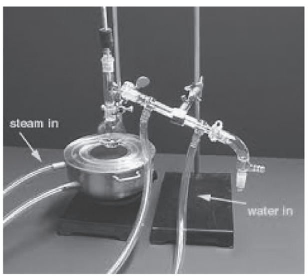
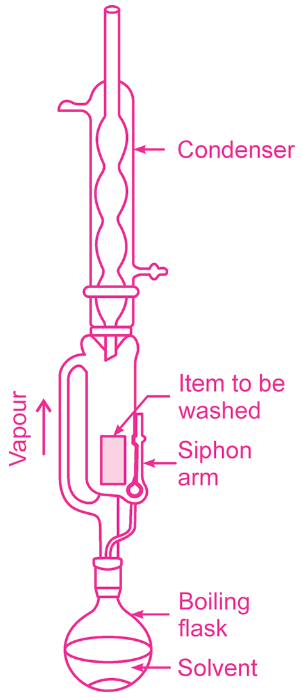
Bunsen burner 	• Supported with tripods and ceramic centred gauze. • Flame should be turned on after ensuring the absence of any fire hazard in the surrounding.
Electrically heated water bath 	It is useful for boiling liquids with boiling point less than 100 °C.
Steam bath 	Steam-heated devices are generally preferred whenever temperatures of 100° C or less are required.

Table ***Contd...***

Electric heating mantle 	It is also known as isomantle, which supplies heat to the containers and flasks. The heating element is insulated and hence the containers can be directly placed on the mantle. Heating mantle with Rheostat is useful for maintaining the intended temperature. It distributes the heat evenly over the surface of the flask and produces no/less harmful hotspots.
Oil bath 	Useful in temperature above 100 °C. The following oils are useful in the heating procedures. - *Hard hydrogenated cotton seed oil:* For temperature up to 250 °C, more advantageous because it is clear, non sticky liquid and solidifies on cooling. - *Medicinal paraffin:* For temperature upto 220 °C. - *Glycerol and dibutyl phthalate:* For temperature up to 140-150 °C. - *Silicone fluids:* Silicone fluids are polymers of inorganic compounds and not derived from petroleum and organics, hence posses unique properties. - (e.g., polydimethylsiloxanes, methylphenyl silicone fluid).

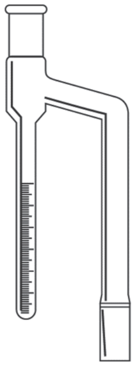
Soxhlet Extractor

Soxhlet extraction is effective in the separation of desired compound with limited solubility than the impurity. Simple filtration is suitable for the separation, if the desired compound has significant solubility in the solvent.



Soxhlet Extractor

SPECIAL APPARATUS

Dean-Stark Apparatus 	It is also known as distilling trap used in the continuous removal of liberated water from the reflux mixture (azeotropic distillations). Solvent vapours along with water escapes in to the condenser and then drip into the trap. The two immiscible liquids gets separated into two layers, the high density water flow to the collector through the side arm and low density liquid flows back into flask.
Kipp's Apparatus 	It is also known as Kipp's generator and useful in the preparation of small volume of gases. In general they are made up of glass and polyethylene. The apparatus is made up of three vertically stacked cylinders and the solid material which generates gas will be placed in the middle cylinder. The top cylinder will be loaded with acid which in turn connected to the middle and bottom cylinder through the tube. The middle cylinder has separate tube with stopcork to collect the evolved gas. The closure of the stopcock will raise the pressure of the gas which expels the acid back to the top of the cylinder.

1.4 Glassware Cleaning

Glassware can be cleaned by immersing them in dichromate solution (cleaning solution), followed by draining and rinsing with distilled water.

Preparation of dichromate solution: Dissolve 5 g of potassium/sodium dichromate in 5 mL of water and cool. Add 100 mL of sulphuric acid slowly with stirring (exothermic reaction, hence use fume cupboard).

- Glasswares can be rinsed with acetone and then with petroleum ether or diethyl ether to remove traces of impurities prior to use.
- Alcoholic solution of sodium hydroxide (120 g of sodium hydroxide in 120 mL of water, dilute to 1000 mL with ethanol) can be used for cleaning polyethylene ware.

1.5 Purification Methods

Common methods employed in the purifications are:

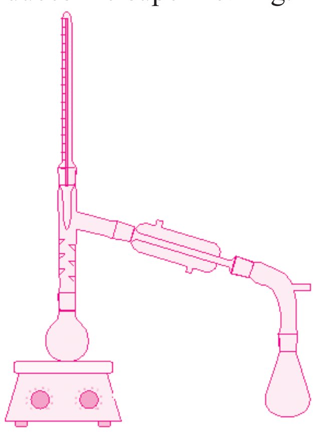
- Solvent extraction
- Washing
- Distillation
- Recrystallization
- Sublimation
- Chromatography
- Drying

Solvent Extraction: Organic compounds can be separated from inorganic impurities by shaking the reaction suspension with suitable immiscible solvents (benzene, carbon tetrachloride, diethyl ether, etc). Evaporation of organic layer (containing organic compound) leaves the residue. Addition of electrolytes (ammonium sulphate, calcium chloride and sodium chloride) ensures the organic layer separation and increases organic extraction.

Washing: Phenol impurities can be removed by washing with sodium hydroxide. Organic bases can be removed by adding dilute hydrochloric acid/sulphuric acid. Unsaturated hydrocarbons of alcohols and ethers can be removed by adding concentrated sulphuric acid.

Distillation: Distillation is the method of separating mixtures based on the differences in volatility of components in a boiling liquid mixture.

Simple and fractional distillation: Non-volatile compounds can be separated by simple distillation. Liquids and low melting point solids (volatile compounds) can be purified by fractional distillation. Distillation apparatus consists of distillation flask fitted with fractional column which in turn attached to condenser and thermometer. In case of simple distillation column is not necessary and flask can be attached directly to condenser. Adding boiling chips to the flask reduces the super heating.



Distillation unit

Upon heating, the contents of the flask vaporizes, passes through the column and condenses back to flask. The vapour passing into the condenser is lowest boiling component of mixture. In fractional distillation the initial and final fractions (lower and higher boiling point components respectively) has to be rejected. Stir the mixture mechanically, in case of suspended insoluble materials present in the mixture to be distilled.

- ***Vacuum distillation:*** Distillation under reduced pressure (lower than atmosphere) is denoted as vacuum distillation. Thermolabile, sensitive and high boiling point liquids can be purified by vacuum distillation.
- ***Steam distillation:*** Temperature sensitive materials (natural aromatics) can be purified by this method.
- ***Isopiestic/isothermal distillation:*** Metal free solutions of volatile acids and bases can be obtained by this method. This can be effected by placing distilled water and solution to be purified in separate beaker in sealed desiccators at room temperature for several days.

Recrystallization: Purification of impure crystalline compounds by crystallization from a suitable solvent or mixture of solvents is known as *recrystallization*. Recrystallization is the effective method for the purification of organic solid compounds. A solid product prepared may contain impurities, and recrystallization separates impurity from product. In this process, the compounds will be crystallized based upon differences in their solubility in a given solvent or mixture of solvents.

The process usually involves following steps:

(a) Dissolving the crude substance in a suitable solvent at its boiling point

(b) Filtering the hot solution from insoluble matter.

(c) Allowing the hot solution to cool. This allows the dissolved substance to crystallize out.

(d) Separating the crystals from the mother-liquor (supernant solution).

The resulting solid is dried and tested for purity by checking its melting point. This process is repeated until pure compound is obtained which is evidenced by its unchanged melting point.

Simplified general procedure for the purification of impure solids

1. Select the right solvent based on the solubility of sample (trial and error method).
2. Place beaker containing known amount of solvent on hot plate.
3. Add small amounts of boiling solvent to the impure solid (partially soluble in solvent) taken in the beaker.
4. Dissolve the solid by heating and stirring if necessary (add more solvent, if necessary to obtain saturated solution).
5. Warm the stemless funnel with the vapours of solvent and filter the hot solution through fluted filter paper placed in funnel.

6. Dissolve the crystals if any formed with adding additional amount of solvent.
7. Cool the clear liquid (collected in the beaker) to room temperature.
8. Place the beaker in an ice-bath (do not disturb) and filter the crystals formed through Buchner funnel (previously wet with solvent).

Steps involved in recrystallization process

- Selection of solvent
- Solubilization of the compound
- Decolorization
- Impurities filtration
- Crystallization
- Filtration
- Drying

Selection of solvent: Solvent can be one or mixture of two liquids. In general solvent with partial solubility will be selected for recrystallization. Solute should be insoluble at room temperature and soluble in high temperature (boiling point). A solvent that can completely dissolve a solid in hot condition gives crystals when the temperature is low. Soluble impurities stay in solution and some of the pure compound may also remain dissolved.

The following are the desired features of a solvent for recrystallization

- It must have high solvent power at elevated temperature and comparatively low power at laboratory temperature.
- It should dissolve the impurities readily to a very small extent.
- It should yield well formed crystals of the purified compound.
- It should not react chemically with the substance to be purified.

If two or more solvents are equally suitable for recrystallization, then the selection depends upon ease of manipulation, toxicity, flammability and cost. If the substance is found to be far too soluble in one solvent and much too insoluble in another solvent to allow of recrystallization, *mixed solvents* or *solvent pairs* may be frequently used. In this case, recrystallization is carried out near the boiling point of the mixture.

Mixture of solvents also can be used when single solvent is not applicable. Second solvent can be added to reduce the strength of first solvent. Most common mixture includes alcohol (strong solvent) and water (weak solvent). For polar molecules (containing oxygen and/nitrogen atom) alcohol-water or alcohol-pentanone is more suitable.

Most commonly used solvent pairs are

- Alcohols and water
- Alcohols and toluene
- Toluene and light petroleum

- Acetone and light petroleum
- Diethyl ether and pentane
- Glacial acetic acid and water
- Dimethylformamide and water
- Dimethylformamide and toluene

Solubilization of the compound: Sample should be dissolved with the aid of solvent by the slow addition of solvent.

Decolourization: The coloured impurities present along with the product can be removed by the treatment with charcoal and filtration. Coloured impurities will adhere to the large surface of charcoal and can be separated by filtration. This step is not necessary, when there is no coloured impurities present.

Impurities filtration: Insoluble impurities will be removed by the filtration through fluted filter paper (provides larger surface area and increase rate of filtration) and stem less funnel (avoids crystallization in the stem during crystallization).

A simple filtration in hot condition separates the product from insoluble impurities. Filtrate upon cooling crystallizes out the pure product and the soluble impurities remain in the solvent. Thus both soluble and insoluble impurities can be removed to obtain the pure solid product.

Crystallization: Solvent upon cooling and/or evaporation initiates crystallization of the solid product.

Filtration: Solid product which crystallizes out can be collected by simple filtration.

Drying: Separated solid may contain some residual solvent in it, so it requires drying to remove solvent completely.

Recrystallization never offer 100% product due to loss while physical handling, impurities and some amount will remain in saturation with solvent. Excess of solvent, inadequate warming and selection of wrong solvent also reduces the yield (crystallization).

Procedure for the purification of benzoic acid

a) Heat the benzoic acid with enough water to dissolve all soluble material.
b) Filter the hot solution to remove insoluble material (if any).
c) Cool the solution to effect crystallization.
d) Filter, wash with water and dry the product.

Procedure for the purification of benzamide

a) Dissolve finely powdered benzamide in boiling water.
b) Filter the insoluble impurities through filtration.
c) Allow it cool to room temperature and collect the crystals through filtration.
d) Wash the crystals with cold water and dry the product.

e) **Sublimation:** The chemical transformation of solids into vapours is known as sublimation. Volatile solids can be converted into vapours on heating and vapours condense as purified solid on cooling. It helps in the purification of volatile solids like naphthalene, iodine, camphor, etc.

Purification of iodine

- Cover the impure iodine placed in the beaker with glass dish.
- Place ice cubes on the glass dish (cover).
- Heat the beaker gently by keeping over the wire gauze.
- The impure iodine vapourizes leaving the impurities.
- Iodine vapours on cooling sublimes in to solid.
- Isolate the pure iodine from glass dish by scratching.

Chromatography: Chromatography is the collective term for the set of techniques applicable for the separation of mixtures. The mixture to be separated is dissolved in mobile phase which carries it through the stationary phase. Column chromatography and thin-layer chromatography (TLC) are the two important separation techniques useful in organic chemistry laboratories.

Column chromatography: Useful in the separation of large quantities of mixtures.

Thin-layer chromatography (TLC): Thin-layer chromatography is a simple, inexpensive analytical and purification technique.

Thin-layer chromatography for salicylic acid and acetyl salicylic acid (aspirin)

- ***Preparation of sample:*** Dissolve the specified quantity of salicylic acid and acetyl salicylic acid in ethanol separately.
- Separately use these solutions for spotting on the TLC plate.
- ***Preparation of developing chamber:*** Prepare mobile phase (hexane: ethyl acetate: acetic acid, 6.5:3:0.5) and load into developing chamber (solvent tank). Cover the chamber with lid to ensure the saturation of chamber with solvent vapour. Line the inside of the beaker with filter paper wet with the mobile phase; this also saturates the chamber with solvent vapour.
- ***Preparation of TLC plate:*** Prepare the silica gel paste, by mixing 5 gm of silica gel with 30 ml of water. Pour this paste onto the glass plate and spread uniformly. [Alternatively aluminium backed precoated plates can be used.] Handle TLC plates by edges only and avoid touching the surface. Draw a line (light) about 1-2 cm from the bottom of the TLC plate.
- ***Loading the TLC plates:*** Dip the capillary into the sample solution and spot on the plate. Touch the tip of the capillary onto the plate, and allow it to dry. Spot the sample for three times and the size should be in between 0.5-1 mm. Use different capillary tube for each sample.

- ***Developing the TLC plates:*** Place the TLC plate into the chamber and make sure that the mobile phase level is below the baseline present in plate. Solvent rises by capillary action and component present in the sample will rise up. Allow the plate to elute until the solvent front is about 1 cm from the top. The rate of sample elution depends upon their partitioning with silica gel. A more polar component spends more time on the polar silica gel surface, move up more slowly than the non-polar component.
- ***Visualizing the TLC plates:*** Remove the plate, mark the solvent front and allow it to dry. Visualize the spots of TLC plate by placing in a UV chamber and keeping in iodine chamber. Different components will appear at distinct locations, and pure sample produces single spot.

  Note: UV light is harmful to eyes and skin, so don't expose hands or eyes to UV light.
- ***Calculation of Retention factor* (R_f):** The ratio of the distance travelled by the sample and distance travelled by the mobile phase (solvent front) is known as R_f.

$$\text{Retention factor } (R_f) = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

1.6 Drying

Removal of solvents: Removal of solvent from recrystallized compounds can be effected by heating them in an oven above the boiling point of the solvent followed by cooling in a desiccator.

Removal of water: Thermal stability of the compounds determines the method of choice. Direct distillation is suitable for removing organic liquid from solids and liquids. During distillation, guard tube with calcium chloride/silica gel prevents the entry of moisture. Substances with melting point above 100 °C and stable in air can be dried using hot air oven. Drying compounds in vacuum desiccator over sulphuric acid, phosphorous pentoxide, silica gel, calcium chloride and similar desiccants is the safest method of drying water. Water in gases can be removed by adsorption on to a drying agent.

Desiccator

Most common desiccants

- ***Calcium sulphate (anhydrous):*** Very efficient and suitable for most of the organic compounds.
- *Magnesium sulphate (anhydrous).*
- *Sodium sulphate (anhydrous).*
- ***Calcium chloride:*** Absorbs water and becomes hexahydrate below 30 °C (slow in action and less efficient). It is suitable for drying aryl halides, esters, ethers and hydrocarbons. It is unsuitable for drying alcohols, amines, amides, amino acids, ketones and phenols.

- ***Calcium oxide (anhydrous):*** It is suitable for drying alcohols and amines, unsuitable for acids and esters.
- ***Copper (II) chloride (anhydrous):*** It is suitable for esters and alcohols.
- ***Potassium carbonate (anhydrous):*** It is suitable for salting out water soluble alcohols, amines and ketones and unsuitable for phenols, thiols and related compounds.
- ***Granulated silica gel:*** Physical adsorption is the mechanism, which is efficient in room temperature.

1.7 Determination of Melting Point

Melting point is the important physicochemical characteristic of molecules, which can be used for identification purpose. The hydrogen bonding nature of the molecules influence their melting point.

***Definition*:** The temperature at which the highly ordered arrangement of particles in a solid gets converted to more random arrangement that characterizes a liquid is known as melting point. Melting occurs over a temperature range and temperature at which first tiny drop of liquid appears is termed as melting point.

Determination of melting point for organic compounds

Sample filling:

1. Grind the sample, whose melting point has to be determined into fine powder.
2. Seal one end of capillary tube (sealed tubes also can be used). Fill 2-3 mm level of capillary tube with fine powder of sample.
3. Drop the capillary tube through 1 meter length glass tube (broken burette or condenser can be used) several times.

Procedure:

1. Place the capillary tube filled with sample in the melting point apparatus.
2. Switch on the instrument to heat the sample until the contents of the tube melts.
3. Use the knob to set rise of temperature per minute (best set at 2 °C per min).
4. Appearance of first tiny drop indicates the melting of sample and the temperature range should be noted as melting range.
5. Change in crystalline structure, wet appearance and shrinkage of the sample should not be interpret as melting point.

Determination of melting point of mixture of compounds

Sample filling 1: For pure product

1. Grind the pure (recrystallized) sample, whose melting point has to be determined in to fine powder.

2. Seal one end of capillary tube (sealed tubes also can be used). Fill 2-3 mm level of capillary tube with fine powder of sample.
3. Drop the capillary tube through 1 meter length glass tube (broken burette or condenser can be used) several times.

***Sample filling 2:* For mixture of pure and impure products**

1. Grind the pure (recrystallized) and impure products, whose melting point has to be determined into fine powder.
2. Seal one end of capillary tube (sealed tubes also can be used). Fill 2-3 mm level of capillary tube with fine powder of sample.
3. Drop the capillary tube through 1 meter length glass tube (broken burette or condenser can be used) several times.

Procedure:

1. Place the capillary tubes filled with pure sample and mixture separately in the melting point apparatus.
2. Switch on the instrument to heat the sample until the contents of the tube melts.
3. Use the knob to set rise of temperature per minute (best set at 2 °C per minute).
4. Appearance of first tiny drop indicates the melting of sample and the temperature range should be noted as melting range.
5. Change in crystalline structure, wet appearance and shrinkage of the sample should not be interpreted as melting point.

Note:

- The determined melting range may differ (small extent) from literature value, if the solid is not completely pure.
- Rapid heating of melting point apparatus and error in the thermometer may also cause to this.

1.8 Determination of Boiling Point

Boiling point reflects the physicochemical characteristic of molecules and can be used for identification purpose. Boiling occurs when the thermal energy of the particles (due to external temperature) is great enough to overcome the cohesive forces that operated in the liquids. Similar to other physical constants of the matter (melting point and solubility) it also depends on nature of bonds in the molecules. The pressure exerted by the surface of the liquid also decides the boiling point of molecules. The atmospheric pressure has the influence on the surface pressure. The presence of volatile impurities increases the boiling point of liquid.

Liquids whose molecules held by hydrogen bonds are known as associated liquids. The energy required to break these bonds will be more, hence their boiling point will be more than others. Among associated liquids, stronger the hydrogen bond, greater will be their boiling point.

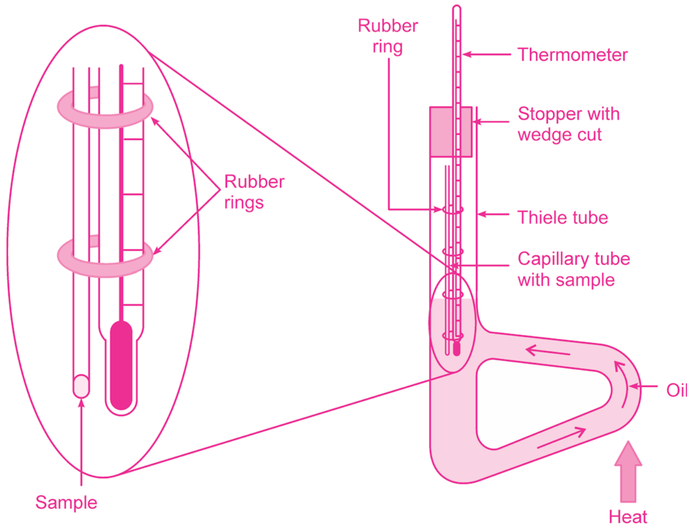
Definition**:** The temperature at which the vapour pressure of the liquid equal to the atmospheric pressure (760 mm) is known as boiling point.

Determination of boiling point for organic compounds (Thiele tube method)

Procedure:

1. Fill a Thiele tube with mineral oil (Liquid paraffin) up to triangle structure.
2. Clamp the Thiele tube in the iron stand placed in the fume cupboard.
3. Insert a Thermometer into the Thiele tube, through a rubber cork.
4. Attach small rubber vial (Durham tube) to the middle of the thermometer bulb with the help of rubber band.
   (a) The rubber band should be on the top, so that its contact with the liquid paraffin bath can be avoided.
   (b) Fill half of the vial with the liquid sample (oil may expand upon heating).
5. Insert the sealed capillary tube into the vial containing sample (open end down and sealed end on the top).
6. Lower the test tube and thermometer set up into a liquid paraffin bath (Thiele tube).
   (Thermometer should not touch the sides)
7. The height of sample should be in the middle of thermometer bulb.
8. Heat the oil gently (oil rises to the trianglular portion of the tube).
9. Continue heating until a stream of bubbles comes out of the capillary tube (individual bubbles can be distinguished.
   (indicates that the vapour pressure inside the tube greater than the atmospheric pressure)
10. Note down the temperature, when there is continuous evolution of bubbles from the capillary tube (**a**).
11. Disconnect the heat supply, and allow the system to cool.
12. Note down the temperature when the evolution of bubbles stops at the end of the capillary tube (**b**).
13. Consider the mean of **a** and **b** as boiling point of the given liquid.

Note: Concentrated sulphuric acid can be used for the liquids with boiling point greater than 200 °C.



1.9 Yield Calculation

Theoretical yield: The maximum amount of product that results from a chemical reaction is called as theoretical yield. The theoretical yield is based upon the molar quantities of the reactants used. It can be calculated from stoichiometric factors derived from the coefficient of the products and reactants.

Practical yield: The actual yield of the product obtained in the chemical reaction.

Relative yield: The ratio between the practical and theoretical yield is known as relative yield. It is also known as fractional yield. It measures the effectiveness of the synthetic procedure.

$$\text{Relative yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}}$$

Theoretically, the yield of a chemical reaction would be 100%. But in practical it is impossible to achieve. Several experimental factors limit the yield (reaction). **Percentage yield:** The percentage yield of the products can be calculated by using the formula given below.

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Yields can be grouped as follows:

% yield	Description
100	Quantitative yield
>90	Excellent yield
>80	Very good yield
>70	Good yield
>50	Fair yield
<50	Poor yield

The percent yield calculation involves a series of steps

Step 1: Writing a balanced chemical equation for the reaction. The correct ratio of the reactants and the products can be ascertained through the balanced reaction. The stoichiometric coefficients are placed before the reagents and products to equal the number of atoms (or molecules) on both the side.

Unbalanced reaction:

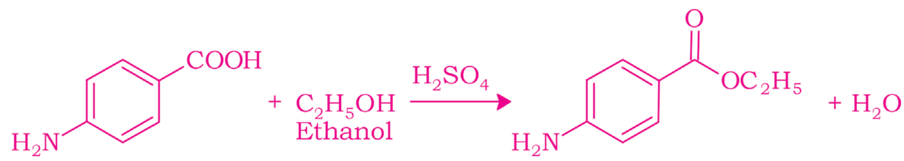
$$\underset{\text{Borax}}{Na_2B_4O_7} + \underset{\text{Sulphuric acid}}{H_2SO_4} + H_2O \longrightarrow \underset{\text{Boric acid}}{H_3BO_3} + \underset{\text{Sodium sulphate}}{Na_2SO_4}$$

Balanced reaction:

$$\underset{\text{Borax}}{Na_2B_4O_7} + \underset{\text{Sulphuric acid}}{H_2SO_4} + 5H_2O \longrightarrow \underset{\text{Boric acid}}{4H_3BO_3} + \underset{\text{Sodium sulphate}}{Na_2SO_4}$$

The differentiation between the reactants (reagents) and other chemical species involved in the reaction are very essential. The chemicals namely, catalysts and solvents are not consumed in the reaction. Hence, they are not considered in the yield calculation.

In case of boric acid preparation, the sulphuric acid is reactant. But in the preparation of benzocaine it acts as a catalyst. Hence it is not considered incase of benzocaine.



para-Amino benzoic acid   Benzocaine

Step 2: Calculate the molecular weight of each reactants and product. The sum of the mass of the atoms generate the molecular weight.

Example, Ethanol – C_2H_5OH

Weight of carbon atom	= 2 × 12.01	= 24.02
Weight of hydrogen atom	= 6 × 1.01	= 06.06
Weight of oxygen atom	= 1 × 16.00	= 16.00
Molecular weight		= **44.08**

The volume of liquid (mL) reactants can be converted into weight (g) by using density parameter.

Step 3: Calculate the moles of a product formed from each mole of reactant. In case of boric acid, four moles of product is formed.

Step 4: *Limiting reagent identification*. The completely consumed reagent in the reaction is known as limiting reagent. It limits the amount of product formed. In case of boric acid preparation, borax is limiting reagent (borax runs out first). The amount of limiting reagent is useful in the calculation of the product yield.

The limiting reagent can be identified through two methods.

Method 1- Reactant mole ratio method: Comparison of mole ratio of the reactants is basis for this method.

Method 2- Product method: This method utilizes the weight of the products. The reactant which produces the lowest yield is known as limiting reagent. The reactants which produce the higher yield is known as excess reagent.

Convert the given information of reactants (volume, weight) into moles. Calculate the mole ratio and compare the calculated ratio to the actual ratio.

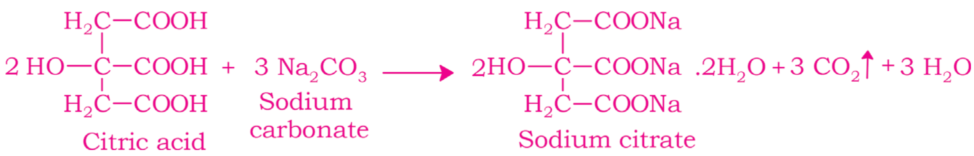
Step 5: *Theoretical yield calculation*. The reaction stoichiometry, provides the information of about the number of moles of product formed with reference to the number of moles of the reactant. Theoretical yiled can be calculated based on the liming reagent concentration.

Step 6: *Percentage yield calculation*. The below given formula is useful in the percent yield calculation.

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Problem example 1: Two mole of citric acid react with three moles of sodium carbonate to produce sodium citrate. Calculate the theoretical yield and percentage yield, if 2 g of citric acid reacts with 2.5 g of sodium carbonate and produces 2.2 g (practical yield) of sodium citrate.

Step 1: Writing a balanced chemical equation for the reaction.



Step 2: Calculate the molecular weight of each reactant and product. The sum of the mass of the atoms generates the molecular weight.

Molecular weight of citric acid = 192.12 g/mol
Molecular weight of sodium carbonate = 105.99 g/mol
Molecular weight of sodium citrate = 294.10 g/mol

Step 3: Calculate the moles of a product formed from each mole of reactant. Convert the mass of reactants in terms of moles.

$$\text{Number of moles of a substance} = \frac{\text{Mass of reactant}}{\text{Molar mass of the reactant}}$$

Number of moles of citric acid = 2 / 192.12 = 0.0104 moles
Number of moles of sodium carbonate = 2.5 / 105.99 = 0.0236 moles

Step 4: Limiting reagent identification. This requires the calculation of theoretical yield of sodium citrate based on the citric acid and sodium carbonate separately.

0.0104 moles of citric acid produces 3.06 g of sodium citrate.

0.0236 moles of sodium carbonate produces 4.62 g of sodium citrate.

Hence, citric acid is the limiting reagent and useful in the percent yield calculation.

Step 5: Theoretical yield calculation.

0.0104 moles citric acid produces 3.06 g of sodium citrate.

Step 6: Percentage yield calculation. It is calculated by the formula given below.

Theoretical yield = 3.06 g

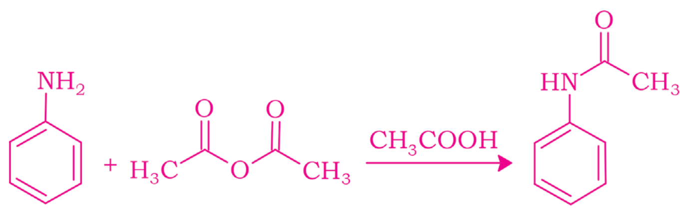
Practical yield = 2.2 g

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

$$= \frac{2.2\ \text{g}}{3.06\ \text{g}} \times 100 = 71.89\%$$

Problem example 2: One mole aniline reacts with one mole of acetic anhydride to produce acetanilide. Calculate the theoretical yield and percentage yield, if 10 mL of aniline reacts with 15 mL of acetic anhydride and produces 11 g (practical yield) of acetanilide.

- ***Step 1***: Writing a balanced chemical equation for the reaction.



- ***Step 2***: Calculate the molecular weight of each reactant and product. The sum of the mass of the atoms generates the molecular weight.

  Molecular weight of aniline is = 93.13 g/mol

  Molecular weight of acetic anhydride is = 102.09 g/mol

  Molecular weight of acetanilide is = 135.17 g/mol
- ***Step 3***: Calculate the moles of a product formed from each mole of reactant. Convert the mass of reactants in terms of moles.

$$\text{Number of moles of a substance} = \frac{\text{Mass of reactant}}{\text{Molar mass of the reactant}}$$

  Number of moles of aniline = 10 × 1.022 (density) / 93.13 = 0.110 moles

  Number of moles of acetic anhydride = 15 × 1.08 (density) / 102.09 = 0.159 moles
- ***Step 4***: Limiting reagent identification. This requires the calculation of theoretical yield of acetanilide based on the aniline and acetic anhydride separately.

  0.110 moles aniline produces 14.86 g of acetanilide.

  0.159 moles acetic anhydride produces 21.49 g of acetanilide.

  Hence, aniline is the limiting reagent and useful in the percent yield calculation.
- ***Step 5***: Theoretical yield calculation.

  0.110 moles aniline produces 14.86 g of acetanilide.
- ***Step 6***: Percentage yield calculation. The below given formula is useful in the calculation.

  Theoretical yield = 14.86 g

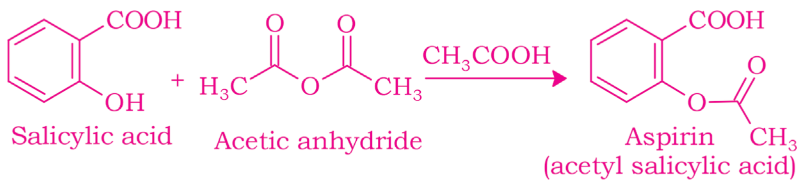
  Practical yield = 11 g

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

$$= \frac{11\ \text{g}}{14.86\ \text{g}} \times 100 = 74\%$$

Problem example 3: One mole salicylic acid reacts with one mole of acetic anhydride to produce aspirin. Calculate the theoretical yield and percentage yield, if 1 g of salicylic acid reacts with 10 mL of acetic anhydride and produces 1.17 g (practical yield) of aspirin.

- ***Step 1***: Writing a balanced chemical equation for the reaction.



- ***Step 2***: Calculate the molecular weight of each reactant and product. The sum of the mass of the atoms generates the molecular weight.
  Molecular weight of salicylic acid is = 138.12 g/mol
  Molecular weight of acetic anhydride is = 102.09 g/mol
  Molecular weight of aspirin is = 180.158 g/mol
- ***Step 3***: Calculate the moles of a product formed from each mole of reactant. Convert the mass of reactants in terms of moles.

$$\text{Number of moles of a substance} = \frac{\text{Mass of reactant}}{\text{Molar mass of the reactant}}$$

  Number of moles of salicylic acid = 1 / 138.12 = 0.0072 moles
  Number of moles of acetic anhydride
  = 10 × 1.08 (density) / 102.09 = 0.106 moles
- ***Step 4***: Limiting reagent identification. This requires the calculation of theoretical yield of aspirin based on the salicylic acid and acetic anhydride separately.
  0.0072 moles salicylic acid produces 1.304 g of aspirin.
  0.106 moles acetic anhydride produces 19.06 g of aspirin.
  Hence, salicylic acid is the limiting reagent and useful in the percent yield calculation.
- ***Step 5***: Theoretical yield calculation.
  0.0072 moles salicylic acid produces 1.304 g of aspirin.
- ***Step 6***: Percentage yield calculation. The below given formula is useful in the calculation.
  Theoretical yield = 1.304 g
  Practical yield = 1.17 g

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

$$= \frac{1.17\ \text{g}}{1.034\ \text{g}} \times 100 = 89.72\%$$