CHE-CC-405

ORGANIC CHEMISTRY PRACTICAL

CBCS-2018

1. **Qualitative Analysis**

i. Identification of unknown organic compounds by general chemical test
ii. Identification of organic compounds of binary mixture by (Thin Layer Chromatography) and determination of R_f value
iii. Purification of organic compounds of binary mixture by Column Chromatography
iv. Characterization of functional group by IR spectra/NMR/Mass

2. **Synthesis of organic compounds:**

i. p- Nitroacetanilide.
ii. p- Nitroaniline.
iii. Ethylbenzoate.
iv. m-Dinitrobenzene.
v. Dibenzyl acetone and its derivatives
vi. Anthranilic acid
vii. Methyl Orange
viii. Adipic acid by chromic acid oxidation of cyclohexanol

3**. Quantitative Analysis**

I. Estimation of Acetyl group
II. Estimation Phenolic group
III. Estimation of Keto group

Book recommended

1) Quantitative and Qualitative analysis By A.I. Vogel
2) Experiments and Techniques in Organic Chemistry, D.Pasto, C.Johnson, & M.Miller, Prantice Hall.
3) Systematic Qualitative Organic Analysis, H. Middleton, Edward Arnold (Publisher).
4) Hand Book of Organic Analysis, Qualitative & Quantitative, M.T. Clarke, Edward Arnold (Publisher).
5) Vogel's Text Book of Practical Organic Chemistry, A.R. Tatchell, John Wiley.
6) Macroscale and Microscale Organic Experiments, K. L. Williamson, D. C. Heath.
7) A Text Book of Practical Organic Chemistry (Qualitative). Arthur I. Vogel.

CHAPTER 1:

Application of TLC and Column chromatography for Separating Compounds

OBJECTIVE

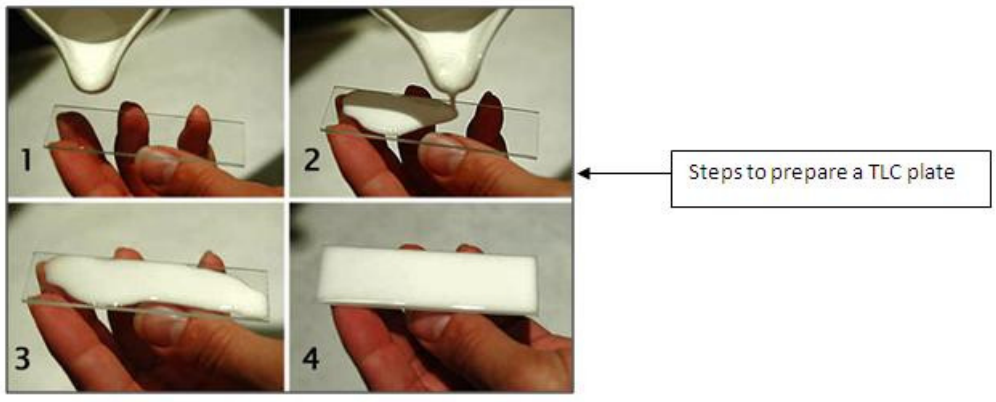
1: Analysis and Separation of mixture of Organic Compounds by TLC and Column Chromatography.

PROCEDURE:

A) Prepare TLC plates (glass/aluminium)
B) Analyse the number of component (uv, stain, I2)
C) Find Rf value
D) Prepare column
E) Separate the compounds
F) Dry and Charaecterise by uv, ir and melting point
G) Laboratory Record Report

PREPARE TLC PLATES (GLASS/ALUMINIUM):

To a 100g of Silica gel (GF 254, SiO_2) in a 200 ml beaker, 10-20 ml of water (or hexane) is added and shacked until a liquid slurry is formed. The above slurry is then poured on a glass plate/aluminium plate (5 cm x 2 cm plate). The palte is kept some time till it dry and make a thin uniform layer of adsorbent. Also there are readymade TLC plates available in shops.



Developing TLC plates using SiO_2 on Glass /Aluminium plate

RUNNING TLC

Now 5-10 mg of the supplied unknown mixture is dissolved in 2-3 ml of Ethylacetate/DCM/ Methanol and other solvent, where it is soluble. Now make a baseline at the base of TLC plate by a pencil marker at a minimum of 0.5-1 cm. Also mark another line at the top end of plate just 1 cm from the end. Now put a spot on 2 to 3 times the base line with the help of capillary by dipping the capillary in the supplied/stock mixture.

In the other hand, prepare the Eluent solution (Mobile Phase) with Pure hexane, 1:1 Hexane and ethylacetate (50 % solution) and prepare also different % of solution (5%, 10%, 20%, 30%, 50% 80%).

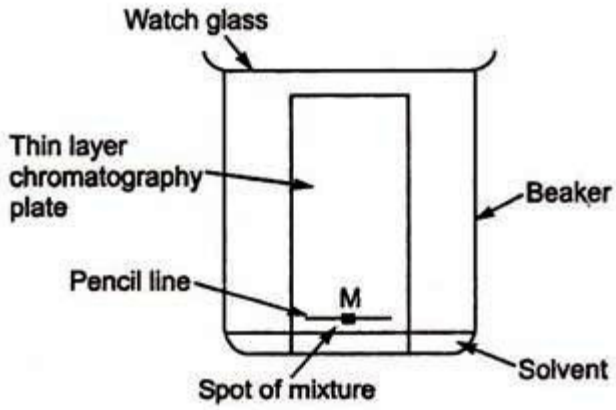
Take about glass beaker and add 0.02 to 0.0 8 cm of solvent in this chamber. Now dip the TLC palte spotted with mixture and dip in the TLC chamber covered with the glass lead. Now wait for some time until the eluent solvent rises up to the 1 cm below the top end of tlc plate.

ANALYSE THE NUMBER OF COMPONENT (UV, STAIN, I_2)

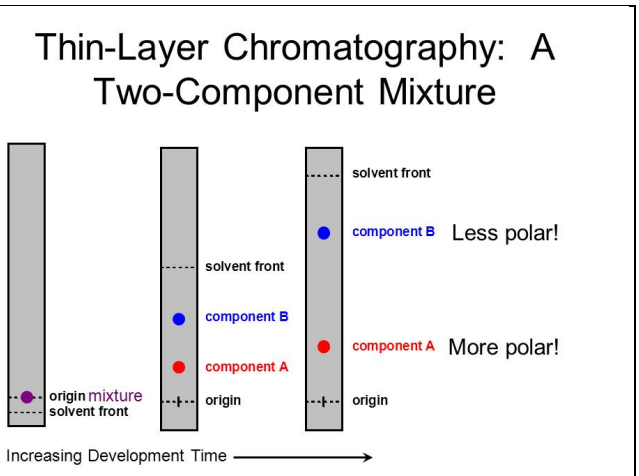
Now take out the plate out of TLC chamber once the solvent reaches the front line. Now visualize the above tlc plate in UV chamber (short and long wave) and count the no. of visible spot and mark the spot by pencil. If the compound is not visible in UV, the plate can be visualized in the Iodine chamber or can be visualized in the TLC stains.

Find Rf value by measuring the distance travelled by solvent in comparison with distance travelled by each component. If separation is not good, then % of eluting solvent is changed and redetected until a good separation is Found.

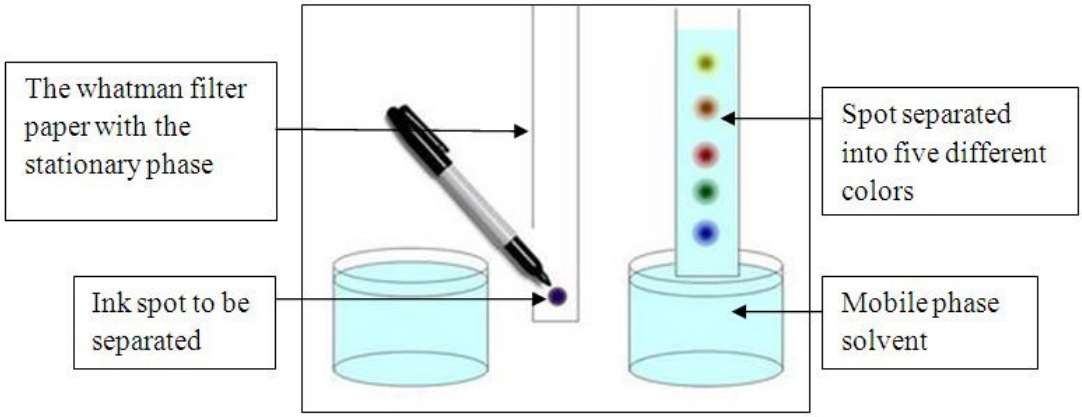
Spotting the Compound



TLC chambers



TLC Chromatograms



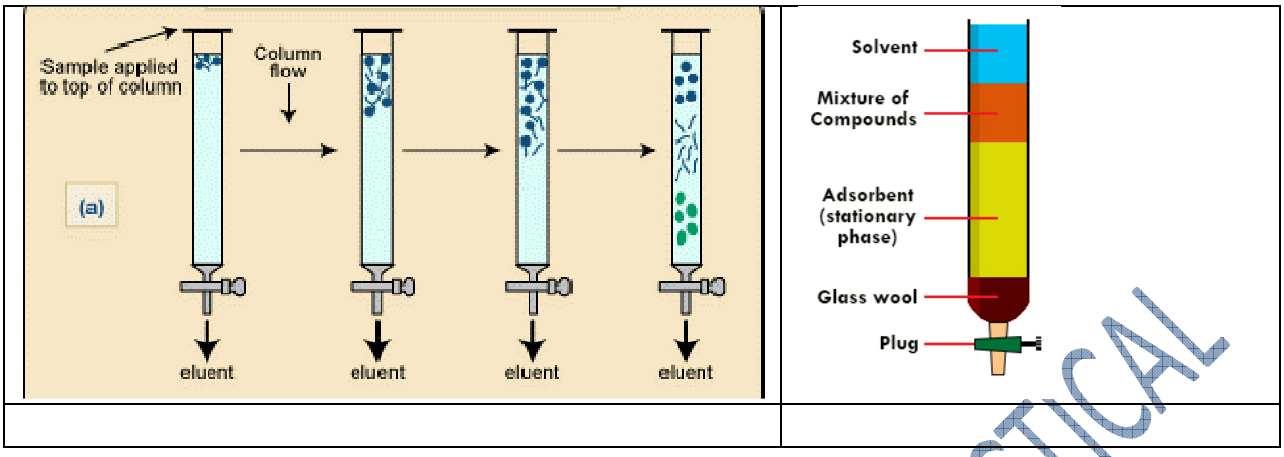
TLC chromatography of a Marker Ink

PREPARING COLUMN AND SEPARATING MIXTURES:

In a typical column, the stationary phase, a solid adsorbent normally silica gel ($_{SiO2}$) or alumina (Al_2O_3), is placed in a vertical glass column. The mobile phase, a liquid, is added to the top of the column and flows down through the column by either gravity or external pressure (flash chromatography). Separation of compounds is achieved through the varying absorption on and interaction between the stationary and mobile phases.

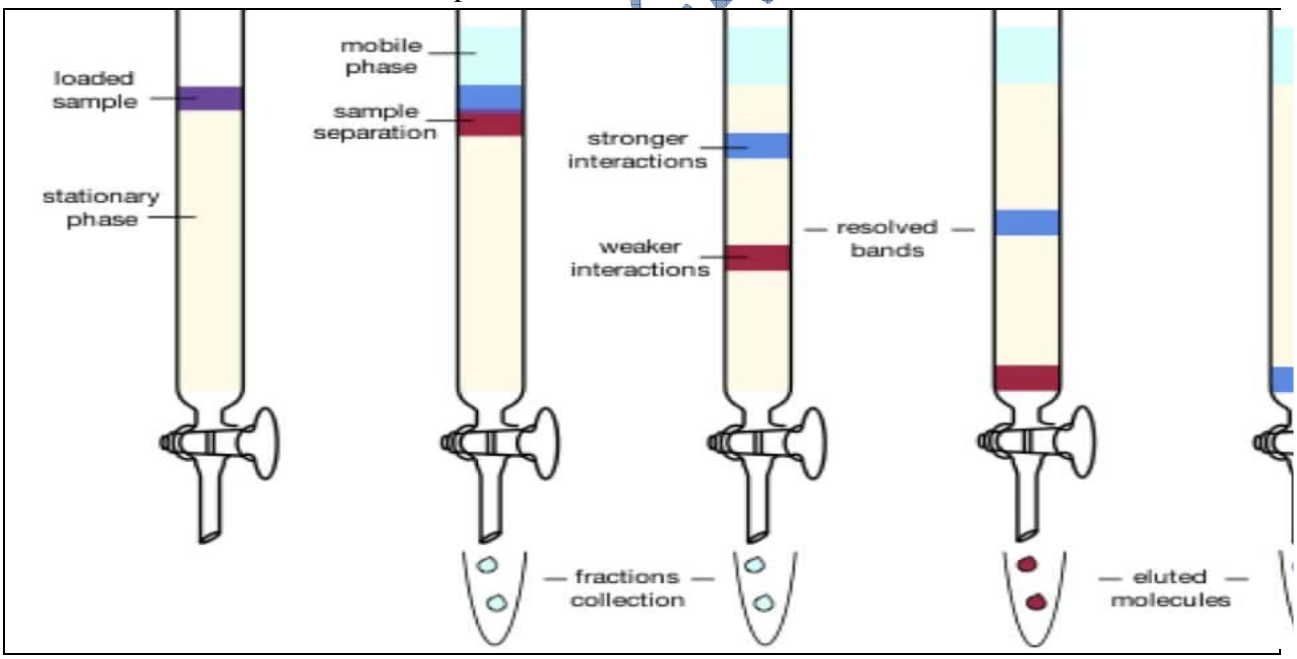
PROCEDURE:

Take a long Glass sintered column (If not sinterd, put a small cotton there at one end). And Fix in a iron stand vertically. Make a slurry in a beaker taking Column silica gel and Hexane solvent. The above slurry is poured in the upper end of column and solvent hexane is eluted with tube collector at end until a with uniform slurry is packed without air bubbles.



Now put the collecting flask/test tubes at the bottom. The supplied mixture is mixed with dry silica gel and dryed and put in the upper part of the stationary phase. Now solvents from lower % to higher percentage is run. Parallelly you can see the different color bandes in the column. Collect the each band in different test tubes/flasks. Check the TLC of all the testtubes and collect same type of TLC spot in a round bottom flask and dry them separately.

Dry the compound with vaccum pump. Take their IR , Melting point and Boiling points. Charracterise the Functional Group.



NOTES AND DISCUDSSION on TLC

TLC Tip 1: Choice of Solvent System (Mobile Phase)

The choice of solvent system is critical in thin-layer chromatography. Follow the guidelines and table below to find the most suitable mobile phase for your separation.

- To choose the right solvent, start with pure solvents of medium elution strength.
- Perform spot tests to compare different solvent systems.
- Single solvents are seldom used in TLC; most solvent systems contain several components, but keep it as simple as possible.
- The solvent system must be capable of wetting the TLC layer.
- Use appropriate solvent purity,Refer to scientific literature or pharmacopoeia monographs to facilitate your search.
- In the table below, the solvents are listed in increasing order of elution strength (according to Halpaap's eluotropic series).

	Solvent		Velocity coefficient, k (mm^2/s)
Lower elution strength	1	n-Heptane	11.4
	2	n-Hexane	14.6
	3	n-Pentane	13.9
	4	Cyclohexane	6.7
	5	Toluene	11.0
	6	Chloroform	11.6
	7	Dichloromethane	13.2
	8	Diisopropyl ether	13.2
	9	tert-Butanol	1.1
	10	Diethyl ether	15.3
	11	Isobutanol	1.6
	12	Acetonitrile	15.4
	13	Isobutyl methyl ketone	9.1
	14	2-Propanol	2.5
↓ **Higher elution strength**	15	Ethyl acetate	12.1
	16	1-Propanol	2.9
	17	Ethyl methyl ketone	13.9
	18	Acetone	16.2
	19	Ethanol	4.2
	20	1,4-Dioxane	6.5
	21	Tetrahydrofuran	12.6
	22	Methanol	7.1
	23	Pyridine	8.0
	Sorbent		TLC plate silica gel 60 F254 Merck

	Type of chamber	N-chamber with chamber saturation
	Room temperature	22 °C
	Migration distance of solvent	100 mm
Source: Applied Thin-Layer Chromatography, Elke Hahn-Deinstrop, page 71		

TLC Tip 2: Choice of TLC Layer (Stationary Phase)

To help you select the optimal stationary phase for your analysis, the table below shows the most popular pre-coated TLC layers available and their typical applications.

Sorbent material	Chromatographic principle	**Typical applications**
Aluminum oxide	Adsorption chromatography due to polar interactions	Alkaloids, steroids, terpenes, aliphatic, aromatic and basic compounds
Cellulose	Depending on acetyl content transition from normal phase to reversed phase chromatography	Anthraquinones, antioxidants, polycyclic aromatics, carboxylic acids, nitrophenols, sweeteners
Kieselguhr	Commonly impregnated for reversed phase separations	Aflatoxins, herbicides, tetracyclines
Silica		
Standard silica gel, also with concentrating zone	Normal phase chromatography	Most frequent application of all TLC layers, Aflatoxins
Silica gel G, impregnated with ammonium sulfate		Surfactants, lipids (neonatal respiratory syndrome)
Silica gel 60, impregnated with caffeine for PAH determination	Charge transfer complexes	Polycyclic aromatic hydrocarbons (PAH) acc. to German drinking water specification
Cyano-modified layer CN	Normal phase and reversed phase chromatography	Pesticides, phenols, preservatives, steroids
DIOL-modified layer		Steroids, hormones
Amino-modified layer NH_2	Anion exchange, normal phase and reversed phase chromatography	Nucleotides, pesticides, phenols, purine derivates, steroids, vitamins, sulfonic acids, carboxylic acids, xanthines
RP layers		
RP-2, RP-8, RP-18		Nonpolar substances (lipids, aromatics)
Silica gel 60 silanized		Polar substances (basic and acidic pharmaceutical active ingredients)
RP-18 W/UV254, wettable	Normal phase and reversed phase chromatography	Aminophenols, barbiturates, preservatives, nucleobases, PAH, steroids, tetracyclines, phthalates
Spherical silica gel		
LiChrospher® Si 60	Normal phase chromatography	Pesticides, phytopharmaceuticals
Source: Applied Thin-Layer Chromatography, Elke Hahn-Deinstrop, pages 22-23		

TLC Tip 3: Pre-Conditioning TLC Plates

Pre-conditioning TLC layers protects them from humidity, which could otherwise diminish their activity and affect chromatogram results.

- A common pre-conditioning method is to place the TLC plate in a development chamber containing highly saturated salt solution with a large amount of undissolved salt, and allowing the plate to condition for several hours. For reproducible results, make sure the solution contains sufficient undissolved salt!
- Other pre-conditioning methods include modifying the TLC layer by exposure to gas, or conditioning the plate with organic solvents, acids or bases.
- During sample application, cover the application area with a clean glass plate to maintain the layer's activity until development is completed.

TLC Tip 4: Correct sample application

The correct sample application on TLC plates is essential for accurate and reproducible separations. Below are a few ways you can avoid errors.

- Record the position of each sample on the data sheet.
- Cross out used lanes to prevent repeated application on any lane, and to ensure that no samples are omitted.
- Avoid applying samples too close to the plate's edge or to the solvent surface.
- Leave sufficient space between application areas.
- Ensure a consistent distance from the bottom edge of the plate for all samples.

TLC Tip 5: Drying TLC Plates

Highly volatile compounds (e.g. α-pinene)

- Dry plates in a cool room to avoid sample evaporation prior to development.

Volatile compounds (e.g. essential oils applied with toluene or n-hexane)

- Dry plates horizontally for a few minutes at room temperature before placing them in the development chamber.

Thermally stable substances (up to 1000 µg/lane from chloroform or methanol)

- Apply uniform heat at a temperature close to the solvent's boiling point for around 20 minutes.

Thermally labile or oxidation-prone samples

- Carry out several drying tests prior to separation.

Important: Keep exposure of plates to blowers as short as possible to protect the layer from airborne dirt particles.

TLC Tip 6: How to Saturate TLC Chambers

TLC development can be performed in saturated or unsaturated chambers. Chromatography in unsaturated chambers results in evaporation of the solvent from the layer, particularly near the front. This leads to higher solvent consumption, and higher R_f values.

Chamber saturation method

1. Line the chamber with strips of filter paper, leaving a gap for observation.
2. Fill the chamber with solvent to a height of 0.5 to 1 cm.
3. Carefully tilt the chamber to moisten the filter paper and equilibrate the chamber with solvent vapors. After a few minutes, the chamber is saturated with vapors.
4. Place the TLC plate in the chamber carefully so that the solvent does not spill over the starting line. Contact between the side of the plate and the filter paper must also be avoided.
5. Development can now proceed.

Learn more about TLC development

See all TLC Tips

TLC Tip 7: Spraying TLC Plates for Derivatization

Safety

- Airborne solvents may be toxic. Wear goggles, gloves and a dust mask while spraying, and ensure good ventilation.
- Avoid chlorinated hydrocarbons (CHC's) to protect yourself and the environment.

Challenges

- Spraying produces a less uniform coating than dipping or in-situ derivatization.
- Difficult to control reagent quantity while spraying.

Recommendations

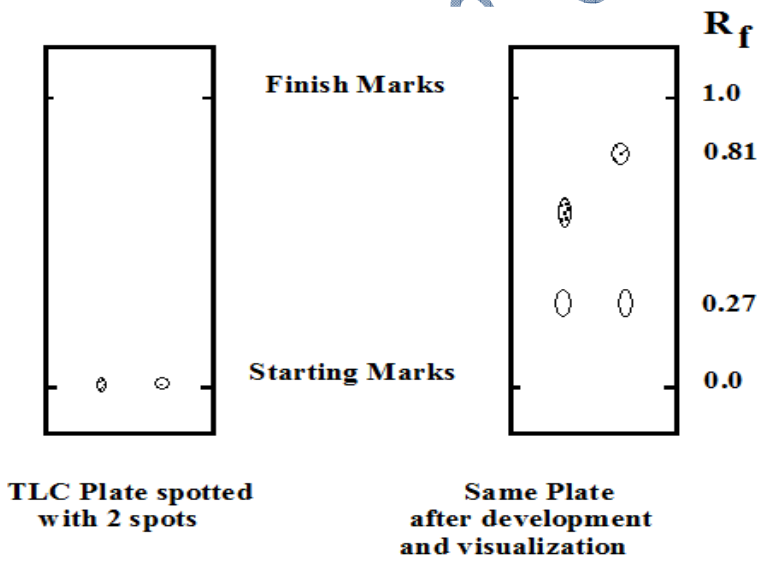
- Always use fresh reagents for each application.
- Reagents stored for long periods should be thoroughly tested prior to usage.

Learn more about TLC derivatization

TLC Tip 8: Quantitative Evaluation with TLC Scanners

- Ensure that all chromatograph lanes are complete before placing the plate in the TLC scanner.
- For accurate analysis of complex sample mixtures, apply the sample as a band (instead of a spot).
- To establish the detection limit, use a blank lane outside the sample lanes for comparison.
- To avoid difficulties with linearity, keep the sample concentration range at a moderate limit.

Caution: Pentane is highly flammable.



Calculate the retention factors for each one of the pigments on your plate.

TLC is a type of planar chromatography.

- It is routinely used by researchers in the field of phyto-chemicals, biochemistry, and so forth, to identify the components in a compound mixture, like alkaloids, phospholipids, and amino acids.
- It is a semi quantitative method consisting of analysis.
- High performance thin layer chromatography (HPTLC) is the more sophisticated or more precise quantitative version.

Principle

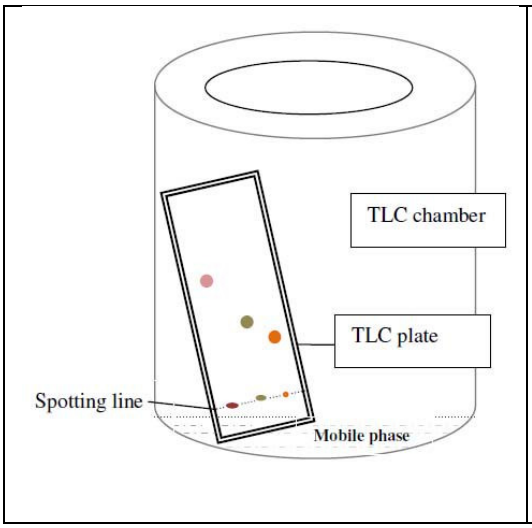
Similar to other chromatographic methods, thin layer chromatography is also based on the principle of separation.

1. The separation depends on the relative affinity of compounds towards stationary and the mobile phase.
2. The compounds under the influence of the mobile phase (driven by capillary action) travel over the surface of the stationary phase. During this movement, the compounds with higher affinity to stationary phase travel slowly while the others travel faster. Thus, separation of components in the mixture is achieved.
3. Once separation occurs, the individual components are visualized as spots at a respective level of travel on the plate. Their nature or character are identified by means of suitable detection techniques.

System Components

TLC system components consists of

1. **TLC plates,** preferably ready made with a stationary phase: These are stable and chemically inert plates, where a thin layer of stationary phase is applied on its whole surface layer. The stationary phase on the plates is of uniform thickness and is in a fine particle size.
2. **TLC chamber.** This is used for the development of TLC plate. The chamber maintains a uniform environment inside for proper development of spots. It also prevents the evaporation of solvents, and keeps the process dust free.
3. **Mobile phase.** This comprises of a solvent or solvent mixture The mobile phase used should be particulate-free and of the highest purity for proper development of TLC spots. The solvents recommended are chemically inert with the sample, a stationary phase.
4. **A filter paper.** This is moistened in the mobile phase, to be placed inside the chamber. This helps develop a uniform rise in a mobile phase over the length of the stationary phase.



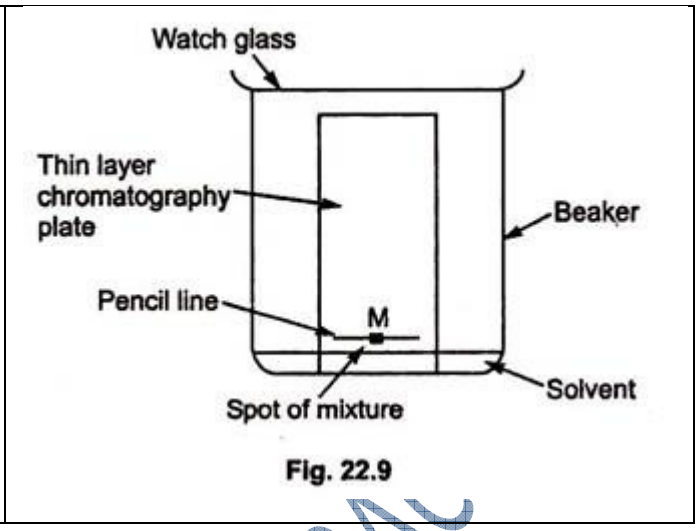


Fig. 22.9

Procedure

The stationary phase is applied onto the plate uniformly and then allowed to dry and stabilize. These days, however, ready-made plates are preferred.

1. With a pencil, a thin mark is made at the bottom of the plate to apply the sample spots.
2. Then, samples solutions are applied on the spots marked on the line in equal distances.
3. The mobile phase is poured into the TLC chamber to a leveled few centimeters above the chamber bottom. A moistened filter paper in mobile phase is placed on the inner wall of the chamber to maintain equal humidity (and also thereby avoids edge effect this way).
4. Now, the plate prepared with sample spotting is placed in TLC chamber so that the side of the plate with the sample line is facing the mobile phase. Then the chamber is closed with a lid.
5. The plate is then immersed, such that the sample spots are well above the level of mobile phase (but not immersed in the solvent — as shown in the picture) for development.
6. Allow sufficient time for the development of spots. Then remove the plates and allow them to dry. The sample spots can now be seen in a suitable UV light chamber, or any other methods as recommended for the said sample.

Advantages

- It is a simple process with a short development time.
- It helps with the visualization of separated compound spots easily.
- The method helps to identify the individual compounds.
- It helps in isolating of most of the compounds.
- The separation process is faster and the selectivity for compounds is higher (even small differences in chemistry is enough for clear separation).

- The purity standards of the given sample can be assessed easily.
- It is a cheaper chromatographic technique.

Applications

1. To check the purity of given samples.
2. Identification of compounds like acids, alcohols, proteins, alkaloids, amines, antibiotics, and more.
3. To evaluate the reaction process by assessment of intermediates, reaction course, and so forth.
4. To purify samples, i.e for the purification process.
5. To keep a check on the performance of other separation processes.

Being a semi quantitative technique, TLC is used more for rapid qualitative measurements than for quantitative purposes. But due its rapidity of results, easy handling and inexpensive procedure, it finds its application as one of the most widely used chromatography techniques.

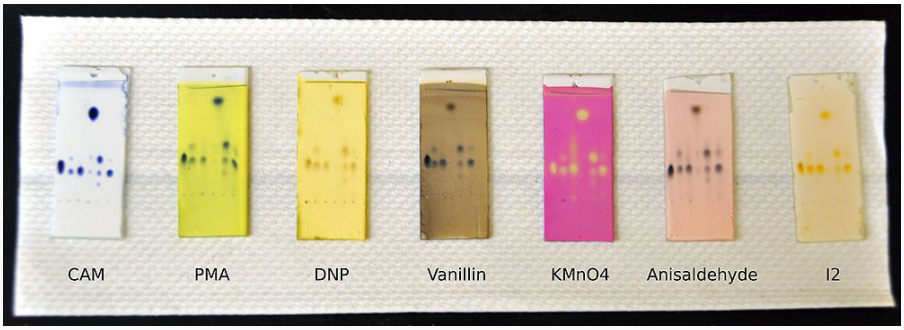
Thin Layer Chromatography Stains-TIPS

For colorless compounds, a visualizing technique is needed to observe TLC results. Stains can be applied by spraying or by dipping of a plate into solution. The latter is by far more convenient. However, in order to work, the right the stain solution SHOULD NOT dissolve analyte spots. For example, permangantate stain is perfect for most not-too-polar organic compounds while acetone-based nynhydrin stain is excellent for amino acids. If analyte solubility in stain solution is inevitable, try to dip the plate as quickly as possible, and then immediately wipe off an excess of stain. Still, there will be some artificial tails added to spots. Also, do not forget, if a compound that must be analyzed is volatile, it may evaporate before the stain visualizes it, especially if heating is required for visualization. The table below represents a few of these techniques:

Name	Application	Preparation
Iodine (I2)	Temporary stain; insert the TLC plate into the chamber and remove it after it develops a light brown color over the entire plate	To a glass bottle with cap (bottle size depends on how much stain you prepare) add 100 g of silica and 5 to 7 g of iodine crystals. Close the cap and shake many times so that iodine is dispersed over the silica.
254nm UV light	UV light excites a fluorescent additive in TLC plate. Compounds screen some of the UV, making fluorescence weaker. Sometimes, visible fluorescence is exited by UV making a spot brighter, and so is colored differently than the background	254nm UV lamp with filter. Darker spots on light green if plates with VU indicator are used. Occasionally brighter spots (typically blue)
p-Anisaldehyde	Carbohydrates; heating required to stain the plate; various colors	Dissolve 18 ml of *p*-anisaldehyde in 540 ml 95% ethanol and cool the solution in an ice/water bath. Mix 30 ml of 97% H_2SO_4 and 6 ml of acetic acid. Cautiously add the acid mixture to the prechilled ethanol solution dropwise at 0°C with vigorous stirring, without splashing. Store the resulting colorless solution in a – 20°C freezer before use.
Bromocresol green	Carboxylic acids yield yellow-green spots on blue background; no heating required	Dissolve bromocresol green (0.08 g) in ethanol (200 ml) to get a clear colorless solution. Slowly add 0.1 N NaOH dropwise until blue color just appears in the solution.
CAM		

	Universal stain; heating required to stain the plate; yields dark blue spots on light background	Slowly add conc. H_2SO_4 (80 ml) to water (720 ml) under stirring followed by ammonium molybdate (40 g) and ceric ammonium sulfate (1.6 g). Stir the resulting mixture to get a clear solution.
Cerium(IV) sulfate [$Ce(SO_4)_2$]	General staining, very effective for alkaloids; should be sprayed on to the plate (not dipped) and then heated for the stain to appear as black spots on yellow-white background	15% aqueous sulfuric acid saturated with cerium (IV) sulfate
Chromic acid	General staining; yields black spots	To a cold (0°C) solution of sulfuric acid (100 ml, 20% v/v aq.), slowly add potassium chromate (2.5 g). Warm the resulting clear bright red/orange solution to room temperature and use directly.
2,4-DNP	Mainly for aldehydes and ketones; yields orange spots, no heating required	Dissolve 2,4-dinitrophenylhydrazine (6 g) in 95% ethanol (100 ml) and add water (40 ml). Stir the resulting mixture to get a clear solution, slowly add conc. H_2SO_4 (60 ml), and stir to get a clear solution.
Dragendorff reagent	Unreactive amines (e.g., carbamate protected amines), alkaloids; yields orange spots, no heating required	Solution A: 1.7 g basic bismuth nitrate in 100 ml water/acetic acid (4:1). Solution B: 40 g potassium iodide in 100 ml water. Mix reagents together as follows: 5 ml A + 5 ml B + 20 ml acetic acid + 70 ml water. Spray plates; orange spots develop. Spots intensify if sprayed later with HCl or 50% water-phosphoric acid.
Ehrlich's reagent	Amines, indole derivatives, antibiotics, steroids; mild heating (lower temperature and shorter heating time; remove the heat source before the background color obscures the spots) required to stain the plate	Dissolve *p*-dimethylaminobenzaldehyde (1.0 g) in 75 ml of methanol and add 50 ml of conc. HCl

Ferric chloride spray	Phenols	Dissolve ferric (III) chloride (1 g) in a mixture of methanol (50 ml) and deionized water (50 ml). Stir the above mixture to get a homogenous solution.
Iodoplatinate (PIP)	Alkaloids	Dissolve hexachloroplatinate (0.5 g) and potassium iodide (10 g) in deionized water (295 ml). To the above mixture add conc. HCl (27 ml). Stir the mixture 4 hr at 0°C.
Morin hydrate	General stain; fluorescently active	Dissolve morin hydrate (100 mg) in methanol (100 g) and stir to get a clear solution
Ninhydrin	Mainly for unsaturated compounds and alcohols; alkenes/alkynes/ aromatics usually stain without heating while other oxidizable groups require heating; yields yellow spots on purple background	Dissolve ninhydrin (1.5 g) in *n*-butanol (100 ml) and then add glacial acetic acid (3 ml). Ethanol can be used in place of butanol.
Potassium permanganate ($KMnO_4$)	Mainly for unsaturated compounds and alcohols; alkenes/alkynes/aromatics usually stain without heating while other oxidizable groups require heating; yields yellow spots on purple background	Dissolve $KMnO_4$ (1.5 g) and K_2CO_3 (10 g) in deionized water (200 ml). To this add 10% NaOH (1.25 ml) and stir to get a clear solution. It will take some time for the solution to clear.
Phosphomolybdic acid (PMA)	Good general reagent; heating required to stain the plate, yields blue–dark green spots	Dissolve 12 g phosphomolybdic acid in 250 ml ethanol.
Sulfuric acid	Heating required to stain the plate; permanent charred spots are produced	5% sulfuric acid in methanol
Vanillin	Good general reagent; heating required to stain the plate, yields a range of colors	To a cold (0°C) clear colorless solution of vanillin (15 g) in absolute ethanol (250 ml), slowly add sulfuric acid (2.5 ml). Warm the resulting clear solution to room temperature and use directly. Store the excess in a refrigerator.



Column Chromatography: TIPS

Column chromatography is a commonly used purification technique in labs across the world. Done right it can simply and quickly isolate desired compounds from a mixture. But like many aspects of practical chemistry, the quick and efficient setting up and running of a column is something that can take years to master. Here we present some of the tips and tricks of the trade to help you set up the perfect column.

In a typical column (Fig. 1), the stationary phase, a solid adsorbent normally silica gel (SiO_2) or alumina (Al_2O_3), is placed in a vertical glass column. The mobile phase, a liquid, is added to the top of the column and flows down through the column by either gravity or external pressure (flash chromatography). Separation of compounds is achieved through the varying absorption on and interaction between the stationary and mobile phases.

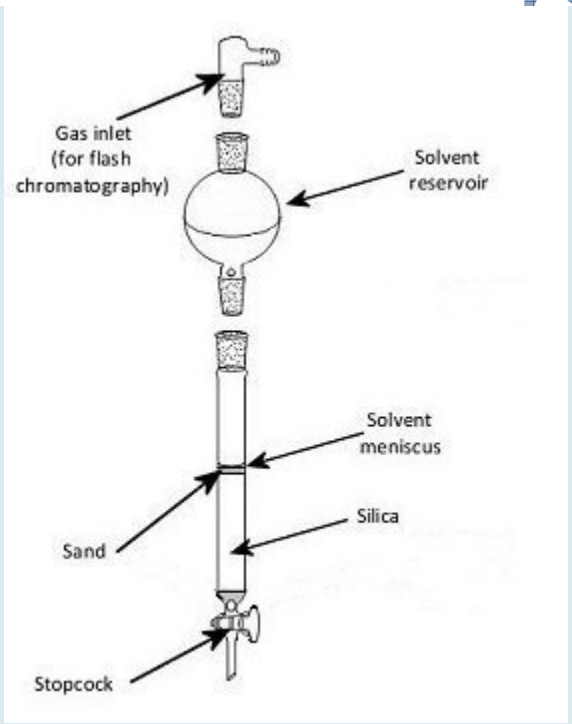


Figure 1. General column set up.

The quality of the separation depends on a variety of factors not least of which is the absence of air bubbles in the stationary phase. To prevent bubbles, the correct packing of a column is important.

1. **Choice of Silica or Alumina for the Stationary Phase**

Silica and alumina are both polar adsorbents so the more polar components in the mixture to be separated are retained more strongly on the stationary phase and are therefore eluted from the column last. Silica is recommended for most compounds, but as it is slightly acidic, it preferentially retains basic compounds. Alumina is slightly basic, so will retain acidic compounds more strongly. It is good for separation of components that are weakly or moderately polar and the purification of amines. Absorbent particle size affects how solvent flows through the column. Silica or alumina are both available in a variety of sizes.

The size is given by the mesh value which refers to the number of holes in the mesh that is used to sieve the absorbent. Thus higher mesh values such as "silica gel 230–400" have more holes per unit area and correspondingly smaller particles than "silica gel 60". Typically, 70–230 silica gel is used for gravity columns and 230–400 mesh for flash columns.

Alumina is available in types I, II, and III. This refers to the water content of the alumina, with I having the least water and III the most. A lower water content means there are more polar sites in the alumina free to bind organic compounds, and polar compounds will remain on the column longer. Alumina of activity II or III, 150 mesh, is most commonly employed.

The techniques for packing a column described below use silica as the stationary phase, but are equally suitable for use with alumina.

2. Preparing the Column

Some columns have glass frits (Fig. 2, **1**) to prevent loss of the stationary phase out the bottom; others do not and will need to be plugged with either glass wool or cotton wool. Which you use is personal preference. Positioning the cotton or glass wool can be awkward at first, but glass frits are harder to clean and may be a source of impurities, such as silica leaking through the frit into the collected fractions. This can be prevented by adding a layer of sand between the frit and the silica. The porosity of frits can also vary. This means that the rate of solvent flow can be different for different columns. Very porous frits will leak more silica, but less porous frits have slower flow rates – sometimes too slow – and can lead to pressure build up in flash chromatography.

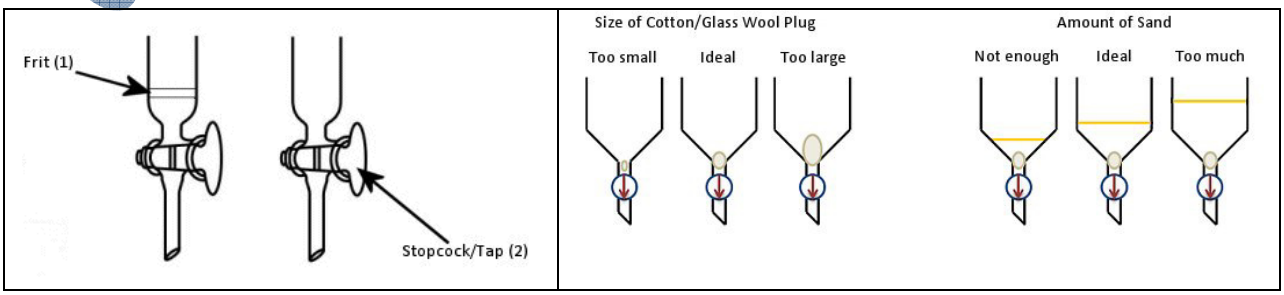


Figure 2. Fritted (left) and non-fritted (right) columns.	**Figure 3.** Guidelines for the correct size of cotton or glass wool and sand for non-fritted columns.

Fritted Column

Find a clean, empty column of suitable size. Clamp the column securely and close the tap or stopcock (Fig. 2, **2**). Add a layer of sand (approx. 0.5 cm, optional).

Non-Fritted Column

The ball of cotton or glass wool should be large enough to plug the bottom of the column, but not so large and densely packed that it restricts solvent flow (Fig. 3). A piece the size of the tip of your little finger should be suitable for most columns. Position the cotton or glass wool ball securely in the narrowest part of the column using a long glass rod or other suitable device. Clamp the column securely and close the tap or stopcock (Fig. 2, **2**). Add a layer of sand until it reaches the main body of the column (approx. 2 cm, Fig. 3). This will give the stationary phase an even base and prevent concentration and streaking of the bands as they come off the column and are collected.

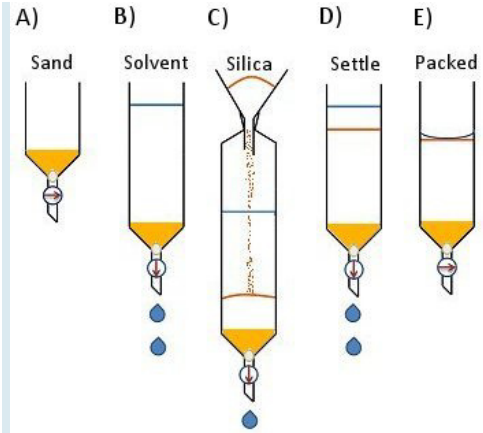
3. Filling the Column

There are several methods for filling columns. You may find one method easier or quicker than the others and always fill a column that way, or you may find that different size columns require different methods. All methods have their pros and cons and you may need to try all three to find the one that you prefer.

Option 1: Dry-Pack Method 1

You will need: Column prepared as in section 2 above; Funnel suitable for dry solids; Something to tap the column with (see box below); SolventSilica or alumina

Method: Fill the column with solvent, allowing some to run through the sand and cotton wool to remove air bubbles (Fig. 4, step B). Place a dry funnel in the top and gently pour the silica or alumina (stationary phase) into the solvent. Allow the solvent to drain to prevent overflowing (Fig. 4, step C). Let the stationary phase settle and gently tap the column (see box below) so that the silica or alumina will pack tightly into the column (Fig. 4, step D).Drain the solvent until the solvent level is just even with the surface of the phase (Fig. 3, step E).



Top Tip: Use rubber vacuum tubing or a cork ring to tap the column and help the silica to settle.

Figure 4. Dry-pack method 1.

Option 2: Dry-Pack Method 2

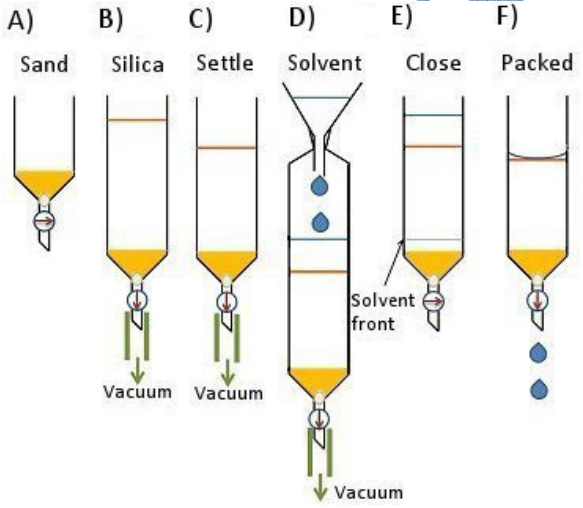
You will need: Column prepared as in section 2 above, Funnel for solvent
Vacuum line, Solvent, Silica or alumina

Method:

Add dry silica gel to the column and apply house vacuum by attaching the vacuum tubing to the bottom of the column (Fig. 5, step B). This will compress the silica gel and keep it compressed for the next steps. Packing can be improved by tapping the column. With the vacuum still applied, pour in the solvent (Fig. 5, step D). Allow the solvent to flow though the column until it is almost at the bottom. At this point, close the stopcock and remove the vacuum line (Fig. 5, step E).

Allow 5–6 columns worth of solvent to flow through the column to ensure complete packing.

Drain the solvent until the solvent level is just even with the surface of the stationary phase (Fig. 5, step F).



Top Tip: If the column drains faster than expected and the solvent level falls below the top of the stationary phase, add more solvent and gently swirl the column to re-suspend the top few cm of silica. Pipette some solvent down the sides to rinse the column and tap gently to remove any bubbles.

Figure 5. Dry-pack method 2.

Option 3: Slurry Method

You will need:

Column prepared as in section 2 above, 2x beakers or conical flasks, Glass rod or Pasteur pipette, Funnel suitable for wet solids, Solvent, Silica or alumina, Pasteur pipette

Method:

Fill the column about one third with solvent (Fig. 6, step B). In a beaker, measure out the required amount of silica or alumina. In a separate flask or beaker, measure solvent approximately one and a half times the volume of silica. Add the silica to the solvent, a little at a time, while swirling. Use a Pasteur pipette or glass rod to mix the slurry. Pour or pipette some of the slurry into the column. Allow the solvent to drain to prevent overflowing (Fig. 6, step C).Tap the column gently to encourage bubbles to rise and the silica to settle (Fig. 6, step D).

Continue to transfer the slurry to the column until all the silica or alumina is added.

Rinse the inside of the column by pipetting solvent down the inside edge.

Drain the solvent until the solvent level is just even with the surface of the stationary phase (Fig. 6, step E).

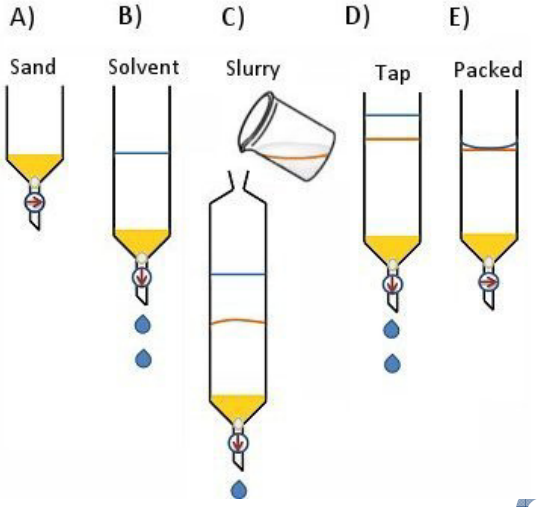


Figure 6. The slurry method.

Top Tip: Use hand bellows to increase the pressure in the column and to force the silica to compact. Repeat several times, rinsing the sides of the column in between, to ensure complete packing.

You are now ready to load your column and isolate the desired compound.

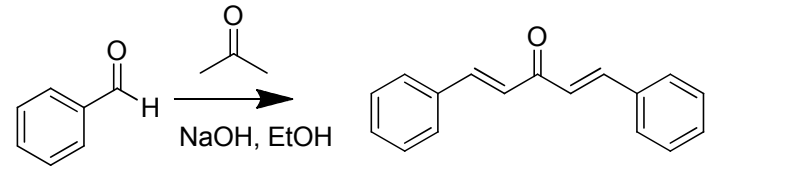
4. Emptying the Column

Once you have your products isolated, all that remains is to empty and clean the column ready for next time. To speed up the process, elute all of the solvent using compressed air and allow air to flow through the column for approximately 2 h. This will give dry, free-flowing silica that is easy to pour into the silica waste container. Alternatively, elute all the solvent and secure the column upside down over a large beaker and allow to dry overnight in a fumehood. Cleaning the column by rinsing with water and acetone is usually sufficient.

Remember! Silica dust is hazardous to inhale. Always handle silica in a fumehood and wear suitable protective clothing. Try to minimize raising dust during handling.

CHAPTER-2:

OBJECTIVE: Synthesis Of Dibenzalacetone

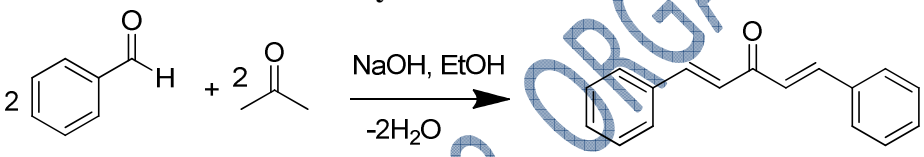


benzaldehyde (1*E*,4*E*)-1,5-diphenylpenta-1,4-dien-3-one

Apparatus Required: Erlenmeyer flask, Buchner funnel, glass funnel, melting point apparatus, UV/Vis spectrometer, FTIR spectrometer

Materials Required : Benzaladehyde, acetone, sodium hydroxide, 95% ethanol, ethyl acetate, ice

Calculation of Stoichiometry:



benzaldehyde (1*E*,4*E*)-1,5-diphenylpenta-1,4-dien-3-one

106.12 59.05 234.29
1 mol 0.5 mol 1 mol

Chemicals	Benzaldehyde	Acetone	Sod. Hydroxide
Formula	PhCHO	CH_3COCH_3	NaOH
MW	106.12	59.05	39.99
Mol	1 mol	0.5 mol	2.5 mol
Wt	1x106 gm	0.5 x 59.06	2.5x39.99
	10.6 gm	2.9gm	10.0g

$$\% \text{ of Yield} = \frac{\text{Theoretical Yield}}{\text{Experimental Yield}}$$

PROCEDURE: A cooled solution of 10 g. of sodium hydroxide (0.25 mol) in 100 ml. of water and 80 cc. of alcohol (Note 1) is placed in a 200ml. wide-mouthed glass jar which is surrounded with water and fitted with a mechanical stirrer. The solution is kept at about 20–25° and stirred

vigorously (Note 2) while one-half of a mixture of (add 5.3 gm out of 10.6 g. 1 mole) of benzaldehyde and 2.9 g. (0.05 mole) of acetone is added (Note 3).

In about two or three minutes a yellow cloud forms which soon becomes a flocculent precipitate. After fifteen minutes the rest of the mixed reagents is added, and the container is rinsed with a little alcohol which is added to the mixture. Vigorous stirring is continued for one-half hour longer, and the mush is then filtered with suction on a large Büchner funnel. The product is thoroughly washed with distilled water (Note 4) and then dried at room temperature to constant weight. The yield is 10.5–11.0 g. (90–94 per cent of the theoretical amount) (Note 5) of a product which melts at 104–107°.

The crude dibenzalacetone may be recrystallized from hot ethyl acetate, using 10 cc. of solvent for each 4.0 g. of material. The recovery in this purification is about 80 per cent; the purified product melts at 110–111°.

1: Find out Rf value of Product using Ethylacetate and Hexane;

$$Rf = \frac{\text{Distance travelled by DBA}}{\text{Distance travelled by Solvent}}$$

2: Find Melting Point of dibenzalacetone

3: Take UV spectra

4: Take IR Spectra

5: Calculate Yield %=

$$\% \text{ of Yield} = \frac{\text{Theoretical Yield}}{\text{Experimental Yield}} = \frac{XX\ g}{23.4} \times 100$$

XX= Amount of experimental yield

2. NOTES

1. Sufficient alcohol is used to dissolve the benzaldehyde rapidly and to retain the benzalacetone in solution until it has had time to react with the second molecule of aldehyde. Lower concentrations of base slow up the formation of the dibenzalacetone and thus favor side reactions which yield a sticky product. Higher concentrations of base give added difficulty in washing. These concentrations were suggested by, and are approximately the same as, those used in the preparation of benzalacetophenone described in Org. Syn. Coll. Vol. I, **1941**, 78.

2. Only temperatures between 20 and 25° were tried; it was assumed that a change of temperature would have the same effect that it has in the preparation of benzalacetophenone mentioned above.

Stirring is essential, as it makes considerable difference in the uniformity of the product.

3. The benzaldehyde was u.s.p. quality which had been washed with sodium carbonate solution and distilled. Commercial c.p. acetone was used. The theoretical quantities are used, since an excess of benzaldehyde results in a sticky product while an excess of acetone favors the production of benzalacetone. The mixture is prepared before addition in order to ensure additions of equivalent quantities.

4. Since the product is practically insoluble in water, large amounts can be used in the washing. Sodium compounds are probably the chief impurities. The dried product contains some sodium carbonate which results from the failure to remove the sodium hydroxide completely. There remain also the impurities insoluble in water. However, the product is pure enough for use in most reactions.

5. If the mush is allowed to stand several hours, chilled, and filtered cold, a slightly larger yield is obtained, but this is not worth while. The filtrate may be used as a medium for a second run in which about 93 per cent of the theoretical yield is obtained. The melting point of the second product is slightly lower.

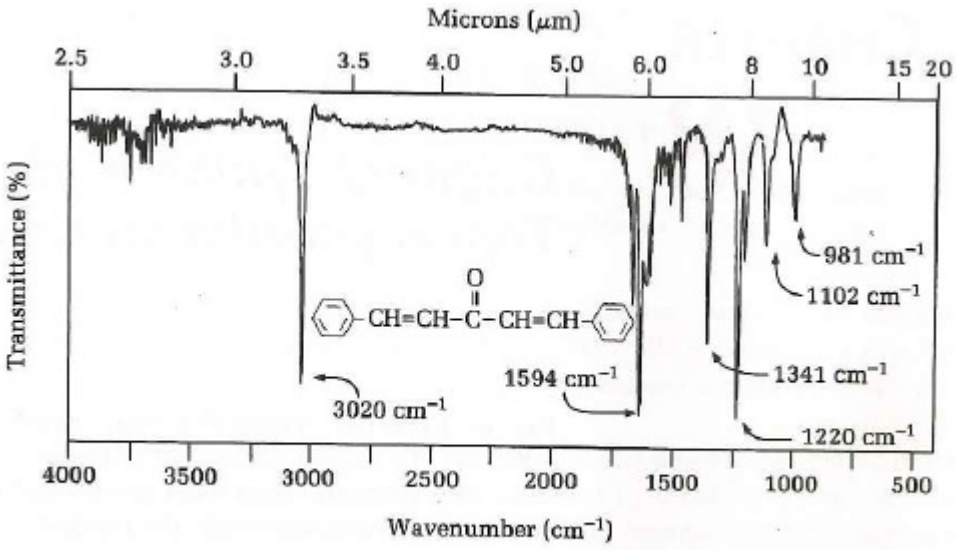
3. DISCUSSION

Dibenzalacetone has been prepared by condensing benzaldehyde with acetone using as condensing agents dry hydrogen chloride,[1] 10 per cent sodium hydroxide solution,[2] and glacial acetic acid with sulfuric acid.[3] It has also been obtained by condensing benzalacetone with benzaldehyde in the presence of dilute sodium hydroxide.[4] Straus and Ecker[5] were the first to record the use of ethyl acetate for crystallization.

References and Notes

1. Claisen and Claparède, Ber. **14**, 350 (1881).
2. Schmidt, ibid. **14**, 1460 (1881); Claisen, ibid. **14**, 2470 (1881); Straus and Caspari. ibid. **40**, 2698 (1907).

3. Claisen and Claparède, ibid. **14**, 2460 (1881).
4. Claisen and Ponder, Ann. **223**, 141 (1884).
5. Straus and Ecker, Ber. **39**, 2988 (1906).



Peaks in IR spectrum 3025 cm^{-1} aromatic, 1649 cm^{-1} C=O 1588 cm^{-1} aromatic

Physical and safety data

substance	Mw (g/mol)	density (g/cm^3)	mp (°C)
NaOH (solved)	40	-	-
Benz-aldehyde	106.1	1.045	-
Acetone	56	0.791	-
Dibenzal-acetone	234.3	-	111-113
Ethanol	46.07	0.79	-114.5

Title: Dibenzalacetone
CAS Registry Number: 538-58-9
CAS Name: 1,5-Diphenyl-1,4-pentadien-3-one
Additional Names: dibenzylidene acetone; distyryl ketone
Molecular Formula: $C_{17}H_{14}O$
Molecular Weight: 234.29
Percent Composition: C 87.15%, H 6.02%, O 6.83%
Line Formula: $C_6H_5CH{=}CHCOCH{=}CHC_6H_5$
Literature References: Prepn from benzaldehyde + acetone: Conrad, Dolliver, *Org. Synth.* **12,** 22 (1939); Haslam, **US 2719863**(1955 to du Pont); Tokár *et al., Acta Chim. Acad. Sci. Hung.* **19,** 83 (1959). Prepn of geometrical isomers: Dinwiddie *et al., J. Org. Chem.* **27,** 327 (1962).

Derivative Type: *trans-trans*-Form
Properties: Crystals from hot ethyl acetate, mp 110-111°. uv max: 330 nm (ε 34,300). Practically insol in water. Slightly sol in alc, ether; sol in acetone, chloroform.
Melting point: mp 110-111°
Absorption maximum: uv max: 330 nm (ε 34,300)

Derivative Type: *cis-trans*-Form
Properties: Light yellow needles from ethanol, mp 60°. uv max: 295 nm (ε 20,000).
Melting point: mp 60°
Absorption maximum: uv max: 295 nm (ε 20,000)

Derivative Type: *cis-cis*-Form
Properties: Yellow oil, $bp_{0.02}$ 130°. uv max: 287 nm (ε 11,000).
Boiling point: $bp_{0.02}$ 130°
Absorption maximum: uv max: 287 nm (ε 11,000)

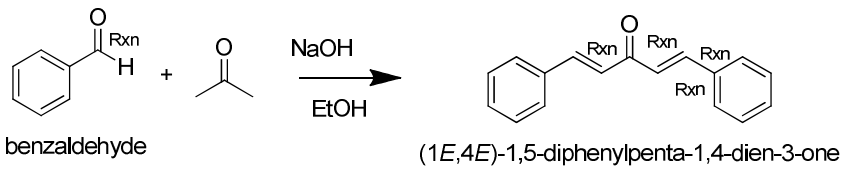
EXPLANATION OF MECHANISM:

Introduction:

The reaction of an aldehyde with a ketone employing sodium hydroxide as the base is an example of a mixed aldol condensation reaction, the Claisen-Schmidt reaction. The double mixed-aldol condensation reaction between acetone and benzaldehyde was carried out. Acetone has α-hydrogens (on both sides) and thus can be deprotonated to give a nucleophilic enolate anion. The alkoxide produced is protonated by solvent, giving a β-hydroxyketone, which undergoes base-catalyzed dehydration. The elimination process is particularly fast in this case because the alkene is stabilized by conjugation to not only the carbonyl but also the benzene. In this experiment, excess benzaldehyde such that the aldol condensation can occur on both sides of the ketone.

Dibenzalacetone is readily prepared by condensation of acetone with two equivalent of benzaldehyde. The aldehyde carbonyl is more reactive than that of the ketone and therefore reacts rapidly with the anion of the the ketone to give a β-hydroxyketone, which easily undergoes base catalyzed dehydration. Depending on the relative quantities of the reactants, the reaction can give either mono- or dibenzalacetone.

Dibenzalacetone is a fairly innocuous substance in which its spectral properties indicate why it is used in sun-protection preparations. In the present experiment, sufficient ethanol is present as solvent to readily dissolve the starting material, benzaldehyde and also the intermediate, benzalacetone. The benzalacetone once formed, can then easily to react with another mole of benzaldehyde to give the desired product in this experiment, dibenzalacetone.



benzaldehyde

(1*E*,4*E*)-1,5-diphenylpenta-1,4-dien-3-one

Reactants					*Products*	
Formula	C_7H_6O	C_3H_6O	HNaO	C_2H_6O	Formula	$C_{17}H_{14}O$
MW	106.12	58.08	40.00	46.07	MW	234.29
Limiting?	***Yes***	No	No	No	Equivalents	
Equivalents					%Completion	
Sample Mass					Expected Mass	
%Weight					Expected Moles	
Molarity					Measured Mass	
Density					Purity	
Volume					Product Mass	
Reactant Moles					Product Moles	
Reactant Mass					%Yield	

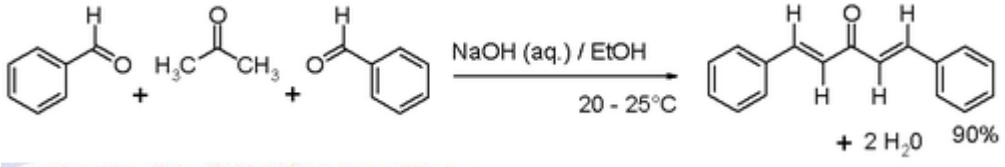
Discussion:

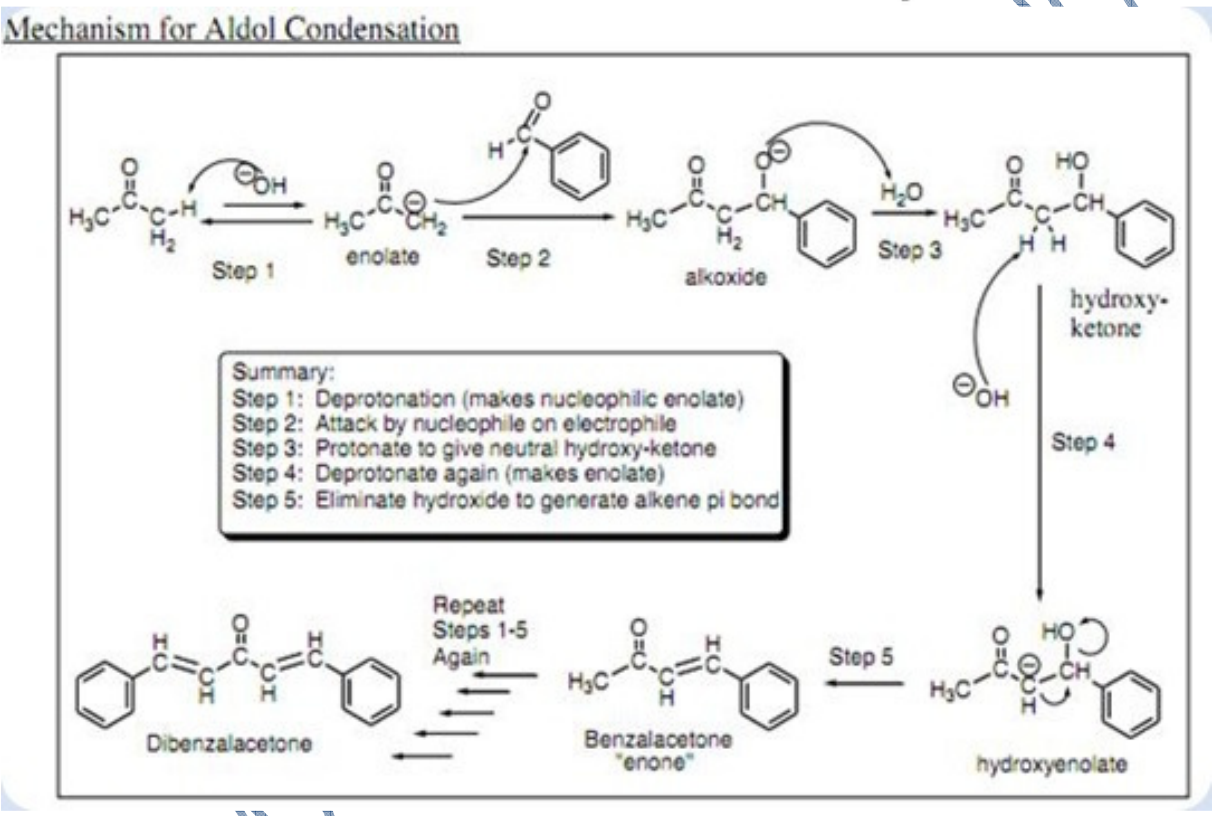
Condensation is a process which joins two or more molecules usually with the loss of a small molecule such as water or an alcohol. Aldol condensation (Claisen-Schmidt reaction) definitely is a process which join two carbonyl groups with a loss of water molecule in order to form β-hydroxyketone. The product is also known as adol because it containing two functional groups which includes aldehyde (or ketone) group and alcohol group. The product dibenzalacetone was formed from the reaction between an acetone molecule and two benzaldehyde molecules. Generally, the aldol condensation is carried out under a base condition.

Sodium hydroxide was mixed with distilled water then was used to react with sufficient ethanol as the first step. The particular reaction is an exothermic reaction which released the heat energy to the surrounding from the reaction. The sodium hydroxide was functioned as a catalyst in the reaction. The ethanol acts as a solvent which allows the acetone and benzaldehyde to dissolve and react with each other. After that, acetone and benzaldehyde were mixed in the solvent which turns to yellow colour quickly. Eventually, the product was formed with a yellow precipitate appear in the reaction after a few seconds. However, there are some impurities and side products were formed in the yellow precipitate. So, recrystallization was carried out by using ethyl acetate as solvent in order to purify the product and hence a pure product could be obtained for the ultraviolet (UV) and IR spectra analysis. In the recrystallization process, the yellow precipitate in ethyl acetate was immersed into an ice-bath in order to obtain a higher yield of product. This is because the heat energy in the precipitate easily to be released since the precipitation formation is an exothermic reaction and hence it maximizes the formation rate of the product.

Acetone is considered as a stable and unreactive compound, so it should be converted into anionic form to increase its nucleophile properties to initiate the reaction. The sodium hydroxide

dissolves in water to produce hydroxide ion and it tends to attack the α-hydrogen in acetone and to form water molecule. The deprotonation of acetone caused the enolate ion was produced as nucleophile which will be used in the synthesis of dibenzalacetone. An enolate ion was formed which it exists as resonance-stabilized structure which shown in the following diagram:



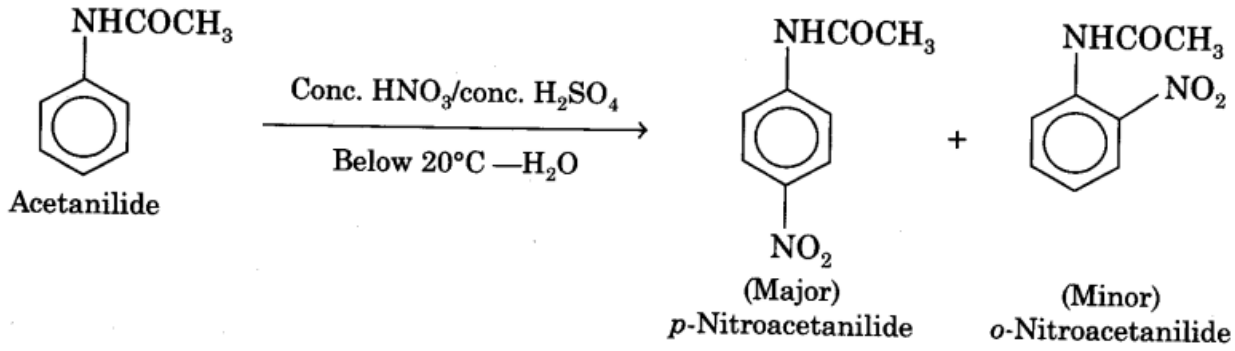


CHAPTER-3:

OBJECTIVE: Preparation of p-Nitro acetanilide from Acetanilide

Theory

The nitration of aniline is difficult to carry out with nitrating mixture (a mixture of cone. H_2SO_4 ,and cone. HNO_3) since —NH_2 group gets oxidised which is not required. So the amino group is first protected by acylation to form acetanilide which is then nitrated to give p-nitroacetanilide as a major product and o-nitroacetanilide as a minor product. Recrystallisation from ethanol readily removes the more soluble ortho-compound and the pure p-nitroacetanilide is obtained. The chemical equation can be written as :



Apparatus

Conical flask (100 ml), beaker (250 ml), measuring cylinder (100 ml), funnel, glass-rod, test-tube, filter-papers, etc.

Chemicals Required

Acetanilide = 5g

Glacial acetic acid = 5 ml

Cone. H_2SO_4 =10 ml

Fuming HNO_3 = 2 ml

Methylated spirit = 20 ml.

Procedure

1. Take a 100 ml conical flask and add 5 g of powdered acetanilide in it. Add 5 ml of glacial acetic acid and stir the mixture by the use of glass-rod.
2. Place 2 ml of fuming nitric acid in a clean test-tiibe and cool it in a freezing mixture (ice + salt) taken in a beaker. Carefully add drop by drop 2 ml of cone, sulphuric acid with constant shaking and cooling.
3. Add the remaining 8 ml of cone. H2S04 drop by drop (with cooling under tap water) to the conical flask containing acetanilide and glacial acetic acid. Place the conical flask in a freezing mixture (Fig). Stir the contents and wait until the temperature becomes less than 5°C.
4. To the cooled contents in the flask add nitrating mixture prepared in step (2) drop by drop with constant stirring. During addition temperature of the mixture should not rise above 10°C. This operation should take about 15 minutes (Fig).
5. Remove the conical flask from the freezing mixture and allow it to stand for 30 minutes at room temperature.
6. Pour the contents of the flask on the crushed ice taken in a beaker. Stir it and filter the crude product. Wash thoroughly with cold water to remove acid.

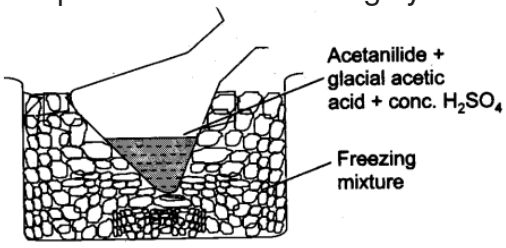


Fig. Flask kept in freezing mixture.

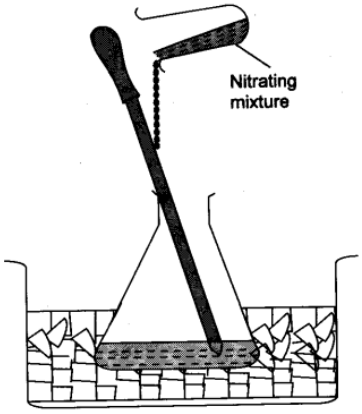


Fig. Preparation of *p*-nitroacetanilide.

7. Recrystallisation of p-nitroacetanilide. Dissolve the crude product obtained above in about 20 ml of methylated spirit. Warm to get a clear solution. Filter while hot and cool the filtrate in ice. o-Nitroacetanilide goes in the filtrate while p-nitroacetanilide is obtained as colourless crystals on the filter paper. Wash the solid on the filter paper with cold water. Dry the solid, weigh it and record its yield.

Result

Weight of p-nitroacetanilide is obtained =……g

Melting point of the compound is…….°C

Note: Approximate expected yield is 4 g.

The melting point of p-nitroacetanilide is 214°C.

Precautions

1. During addition of nitrating mixture, the temperature of the reaction mixture should not rise above 10°C.
2. Addition of fuming nitric acid should be done drop wise.
3. Do not inhale the vapours of nitric acid as they are very corrosive in nature. Addition of nitrating mixture may preferrably be done in a fume-cupboard.

CHAPTER-4:

OBJECTIVE: Estimation of Keto (CO) Group In Acetone

Acetone reacts with iodine in the presence of sodium hydroxide solution to yield iodoform and sodium acetate :

$$CH_3COCH_3 + 3I_2 + 3NaOH = CH_3COCI_3 + 3NaI + 3H_2O$$

$$CH_3COCI_3 + NaOH = CHI_3 + CH_3COONa$$

$$CH_3COCH_3 + 3I_2 + 4NaOH = CHI_3 + CH_3COONa + 3NaI + 3H_2O$$

A dilute aqueous solution of the **sample** is added to a known volume of 1N sodium hydroxide solution, followed by an excess of standard 0.1N iodine solution. After acidification, unreacted iodine is determined by titration with standard 0.1 N sodium thiosulphate solution :

$$1 \text{ Litre } 0.1 \text{ N } I_2 = 1 \text{ Litre } 0.1 \text{ N } Na_2S_2O_3$$

$$= CH_3COCH_3/(6 \text{ X } 10)$$

$$= 0.9680 \text{ g. } CH_3COCH_3$$

Caution: The above procedure is sometimes termed Messinger's method. Aldehydes, compounds which contain an acetyl group, or a group oxidisable by hypoiodite to an acetyl group, interfere ; compounds containing a —CH=CHC=O group *(e.g.,* acrolein or furfuraldehyde) will consume iodine and therefore interfere. Methyl and ethyl alcohols should also be absent.

PROCEDURE

1. a)About 10 ml of Acetone (Reagent grade) was taken in 500ml of volumetric flask. Volume is maked up with distilled water (1^{st} dilution). b) Pipette out 25 ml of this stock solution to second 500ml of volumetric flask. Volume is maked up with distilled water (2^{nd} dilution). c) Again Pipette out 25 ml of this stock solution to second 500ml of volumetric flask. Volume is maked up with distilled water (3^{rd} dilution).
2. Pipette out 25 ml of 3^{rd} this stock solution to second 500ml of volumetric flask.

3. 25 ml. of 1 *N* sodium hydroxide solution was added to the above stock solution, mix well, and allow to stand at room temperature for 5 minutes.

   NB: (Note: To make 1 N **solution**, dissolve 40.00 g **of sodium hydroxide** in water to make volume 1 liter or ***4g in 100ml*** or ***1 gm in 25ml***)

4. Now 50 ml. of 0.1 N **iodine** solution is added with shaking the flask constantly with a swirling motion and Allow the mixture to stand for 15 minutes at room temperature. Pale yellow to brown color will appear.

   NB: (There is a different between iodine solution and iodide solution. Iodide is prepared from KI. but iodine is prepared from both KI and I_2.)

   (For ***iodide solution 0.5M=0.5N*** and for ***iodine solution 0.5M =1N.)***

   (Preparing 0.1 N **iodine** solution: Weigh 40 g of potassium iodide (KI) in a 500 mL glass-stoppered flask and dissolve in 100 mL of purified water. Let the solution come to room temperature, add 12.7 g of resublimed iodine (I_2), restopper the flask, and swirl the flask until the iodine is completely dissolved. Transfer the solution quantitatively to a 1 L volumetric flask, add 3 drops of hydrochloric acid (37% HCl; sp g 1.19) and dilute to 1 L with purified water.

5. Then add 26 ml. of 1N sulphuric acid to make the solution acidic

   NB: Take 6.9 mL of concentrated **sulfuric acid** and diluted it to 250 mL, 0.69 ml of H_2SO_4 in 25 ml H_2O)

6. Now titrate immediately with standard 0.1 N sodium thiosulphate solution to the starch end point as blue color. Note the difference of burette reading. (1.5-2.0 ml will be consumed normally) =V_1

   NB: (Do not add Starch at the beginning, add starch after 1-1.2 ml when the color of mixture is changed from yellowish to white)

   (Preparing 0.1 N $Na_2S_2O_3$, $5H_2O$: dissolve 24.8 g of $Na_2S_2O_3$, $5H_2O$ in 500 ml of freshly distilled water and 2 or 3 drops of $CHCl_3$ or 0.4 g of NaOH and complete to 1000 ml using a volumetric flask.)

7. Run a blank titration, from step 1-6 without adding acetone in step-1 (Only take 25 ml of water in step-a and follow step 2-6) to check the normality of the iodine solution and also to deduce the net volume of standard thiosulphate solution equivalent to the sample.=V_2

CALCULATION

Calculate the percentage of acetone in the sample using the relationship :

1 ml 0.1 N I_2 = 1 ml 0.1 N $Na_2S_2O_3$

= 0.009680 g. CH_3COCH_3

$$\% \text{ Acetone} = \frac{(V1-V2)\times N\times M}{W\times 1000}$$

where

V_1= volume of sodium thiosulphate used in the analysis ;

V_2 = volume of sodium thiosulphate used in blank ;

N = normality of sodium hydroxide solution ;

M =molecular weight of Acetone (58 • 04) ; and

W = weight (g.) of sample. (Known weight)

$$\textbf{No. of Keto (CO) Group} = \frac{(V1-V2)\times M}{W\times 2000}$$

M=Molecular Weight of Acetone=58.08

W= Weight of Sample

Important point regarding Starch Indicator:

A: To **prepare starch indicator solution**, add 1 gram of **starch** (either corn or potato) into 10 mL of distilled water, shake well, and pour into 100 mL of boiling, distilled water. Stir thoroughly and boil for a 1 minute. Leave to cool down. If the precipitate forms, decant the supernatant and use as the **indicator solution**.

B: At the beginning of the titration, due to the relatively high concentration of I_2 in the solution, the colour appears a deep brown/reddish brown (which is in fact due to the presence of $(I_3)^-$ ions which exists in an equilibrium with I_2 and I^-). As the titration proceeds, the concentration of I_2 in solution falls and the colour starts to turn to a lighter shade of brown, then yellow and then very pale yellow.

At the end point of the titration (where no more I_2 is present), the solution should appear colourless. However, the transition from very pale yellow to colourless is not very sharp. At this stage, we add starch which then acts as an indicator of the presence of I_2, the contents of the flask to a deep blue black colour. The colour change from deep blue black colour to colourless is sharp and this makes the end point more easily determined.

The deep blue black colour is due to the formation of a starch-iodine complex. As to why it is added near the end of the titration rather than at the beginning is because the starch-iodine complex at high I_2 concentrations is relatively stable. The release of I_2 from the starch-iodine complex is slow at high I_2 concentrations.

As we are looking for the discharge of the blue black colour, we will end up adding more titrant thinking that the end point has not been reached when in fact, the decolourisation would take place if the iodine was given time to dissociate.

CHAPTER-5:

OBJECTIVE: Estimation Of *Acetyl* ($COCH_3$) Group In Ethylacetate

The hydrolysis of Ester may be conducted with three main types of reagent:

A: Aqueous sodium or potassium hydroxide. This reagent is generally used for esters which are **soluble in water** and are fairly easily saponified. An interesting application is the determination of the acetyl content of the acetate of a polyhydric alcohol; if the molecular weight is known, the number of acetyl groups in the sample can be evaluated. The reaction :

$$R(OCOCH_3)_n + nNaOH = R(OH)n + nCH_3COONa$$

B: ***Potassium hydroxide in diethylene glycol.*** The chief advantage of using **high boiling point solvents** and conducting hydrolyses at their boiling points is that the rate of reaction is increased greatly. Easily saponifiable esters are hydrolysed within a few minutes, whilst difficultly saponifiable ones *{e.g.,* di-n-butyl phthalate) are hydrolysed quantitatively within a reasonable time.

C: ***Alcoholic sodium or potassium hydroxide solution.*** This reagent is used for **esters which are insoluble in water** and which are fairly easily hydrolysed. The alcohol normally employed is absolute ethanol, but for esters that are not hydrolysed readily tsopropanol, »-propanol or n-butanol have been recommended.

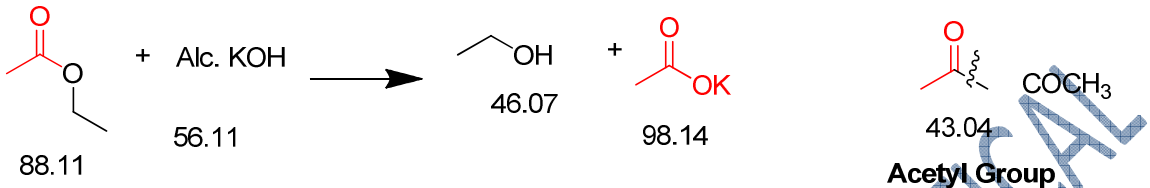
The advantages of the latter are increased speed of saponification due to the higher reflux temperature, their freedom from aldehydes, and the absence of legal restrictions on their sale. It is appropriate to draw attention to possible alcoholysis (ester transposition) with methanolio or ethanolic alkali hydroxide.

When the ester derived from one alcohol is dissolved in another, the second alcohol may replace the first until the reaction attains equilibrium when certain concentrations are reached ; hydrolysis occurs subsequently.

A volatile methyl or ethyl ester can thus be formed by alcoholysis, and it is therefore essential to employ an efficient reflux condenser even when esters of comparatively high boiling point are hydrolysed. Ester transformation explains the experimental fact that methyl, ethyl, n-propyl, n-butyl, iso-amyl and benzyl acetates are hydrolysed at exactly the same rate in methanol.

Ethyl acetate is hydrolyzed in presence of alcoholic KOH to afford alcohol and potassium acetate

$$CH_3COC_2H_5 + \text{Alc. KOH} = C_2H_5OH + CH_3COOK$$



A weighed amount of the ester is heated with a known volume (excess) of the standard alkali hydroxide solution and the excess of alkali is determined, after hydrolysis is complete, by titration with standard acid.

The **saponification equivalent** of an ester is usually defined as the weight of the ester, in grams, which reacts with one gram equivalent of a strong base. The molecular weight of the ester is *a* times the saponification equivalent, where ***a*** is the number of ester groups in the molecule.

Reagents Required: Absolute Ethanol, Pottasium Hydroxide, HCl, Phenolphthalein

PROCEDURE

a) Fit two 250 ml. conical flasks with efficient reflux condensers by means of rubber stoppers

FLASK-1: (With Ethyl acetate) Weigh out accurately about 5 milli-mols of the ethylacetate (440 mg) into one flask. Add 25-0 ml. of 0.5 N alcoholic potassium hydroxide by means of a pipette **(Flask 1)** and add a few small fragments of carborundum (abrasive materials). Boil the flask gently under efficient reflux for 30-40 minutes. Pour 20-25 ml. of water down each condenser, remove the flasks from the condensers, and cool in cold water. Titrate the contents of flask with standard 0.5N or 0.25 Hydrochloric acid, using phenolphthalein as indicator. The end point should be a **faint pink**. Alternatively, titrate the solution until the phenolphthalein is **colourless,** and then back titrate with the original alcoholic alkali solution.

V_1 = volume of 0.5 N HCl solution consumed for netralisation in ml

FLASK-2: (Without Ethylacetate). Add 25-0 ml. of 0.5 N alcoholic potassium hydroxide by means of a pipette **(Flask 1)** and add a few small fragments of carborundum (abrasive materials). Boil the flask gently under efficient reflux for 30-40 minutes. Pour 20-25 ml. of water down each condenser, remove the flasks from the condensers, and cool in cold water. Titrate the contents of

flask with standard 0.5N or 0.25N Hydrochloric acid, using phenolphthalein as indicator. The end point should be a **faint pink**. Alternatively, titrate the solution until the phenolphthalein is **colourless,** and then back titrate with the original alcoholic alkali solution.

V_2= volume of 0.5 N HCl solution consumed for netralisation in ml in Blank

CALCULATION

Calculate the percentage of **Acetyl ($COCH_3$)** in the sample using the relationship :

2000 ml 0.5 N HCl = 2000 ml 0.5 N KOH= Saponification equivalent of ester

$$\% \text{ Saponification equivalent} = \frac{(W)\times 1000}{(V2-V1)\times N1}$$

V_1= volume of 0.5 N HCl solution consumed for netralisation in ml

V_1= volume of 0.5 N HCl solution consumed for netralisation in ml *in Blank*

N = normality of HCl solution ;

W = weight (g.) of sample. (Known weight)

IF, Molecular weight of the sample is known, M=88.12 for ethylacetate

$$\% \text{ Ester} = \frac{(V1-V2)\times M \times 100}{W\times 2000}$$

where

V_1= volume of 0.5 N HCl solution consumed for netralisation in ml

V_1= volume of 0.5 N HCl solution consumed for netralisation in ml *in Blank*

W = weight (g.) of sample. (Known weight)

$COCH_3$=43.04

Then

$$\% \text{ Acetyl} = \frac{(V1-V2)\times 43.04 \times 100}{W\times 2000}$$

$$\text{No. of Acetyl } (COCH_3) \text{ Group} = \frac{(V1-V2)\times M}{W\times 2000}$$

M=Molecular Weight of Ethylacetate=88.12

W= Weight of Sample

Preparation of Alcoholic potassium hydroxide solution (0.5 N); Dissolve 6 g. of A.R. potassium hydroxide pellets in 250 ml. of 95 per cent, ethanol, and allow to settle overnight. Decant or filter the clear solution from any insoluble potassium carbonate. Standardise the solution with standard 0-5. N or 0.25N **hydrochloric acid** or with A.R. potassium hydrogen phthalate, using phenolphthalein as indicator.

Preparing Phenolphthalein as indicator: This a reagent used in the volumetric analysis of weak acids and strong base. Weigh 1g of phenolphthalein powder into 100ml volumetric flask. Add 40ml of 95% ethanol and shake, Make up to the mark with 95% ethanol

Standardizing HCl:

Procedures Weigh about 1.0 - 1.5 g of anhydrous sodium carbonate powder accurately in a watch glass. Transfer the solid totally into a 250 cm3 beaker where about 50 cm3 distilled water is already filled. Wash the watch glass thoroughly by means of a washing bottle and transfer all the washings into the 250 cm3 beaker. Add more water to dissolve the remaining solid. Use a glass rod to stir the solution in order to facilitate the dissolving process. Transfer the solution carefully to a 250 cm3 volumetric flask by means of a filter funnel and a glass rod. Rinse the beaker, glass rod and inner surface of funnel with water and transfer all the washings to the volumetric flask. Repeat this process two or three times. Make up the solution to 250 cm3 in the volumetric flask by adding water just up to the graduation mark. Stopper the flask and invert it about 30 times to mix the solution thoroughly. Pipette 25 cm3 of the sodium carbonate solution into a conical flask, add few drops of **methyl orange indicator** and titrate against the hydrochloric acid solution. Determine the molarity of hydrochloric acid solution.

Colour change : yellow to reddish orange Equation : $Na_2CO_3(aq) + 2\ HCl(aq) \rightarrow 2\ NaCl(aq) + H_2O(l) + CO_2(g)$

Methyl orange indicator

Mix 1 g of **methyl orange** powder with water. Use 2 drops for each 25 mL of solution in a titration.

Notes.

(1) Bark corks must not be used (Use Glass Cork) since the alcohol vapour extracts substances which react with alkali. The rubber stoppers should preferably be warmed with dilute alkali, and then thoroughly washed with distilled water. Ground glass joints may also be used but special precautions must be taken to prevent " sticking ".

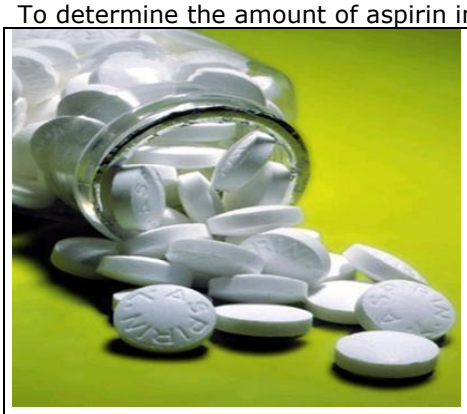
(2) Use the " pipette " weighing bottle shown in Fig. *XIV, 1,* 5, and weigh by difference.

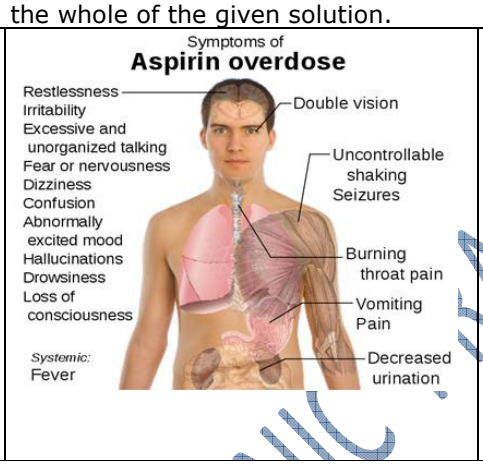
(3) If the condenser is fitted into the flask by means of a ground glass joint, remove the flask from the condenser immediately after the water has been added ; no difficulty will be experienced and no " sticking " or " freezing " of the ground joint should occur.

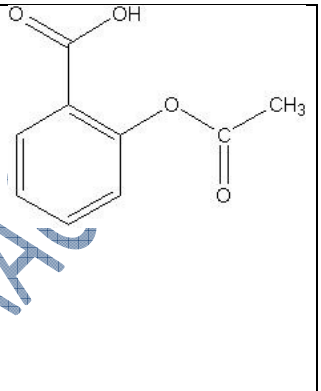
CHAPTER-6:

OBJECTIVE: Estimation of Acetyl Group of Aspirin

- To determine the amount of aspirin in the whole of the given solution.







Introduction:

Charles Frederic Gerhardt, a French chemist was the first to prepare aspirin in 1853. Aspirin is also known as acetylsalicylic acid. It is the acetyl derivative of salicylic acid and is an example of a salicylate drug.

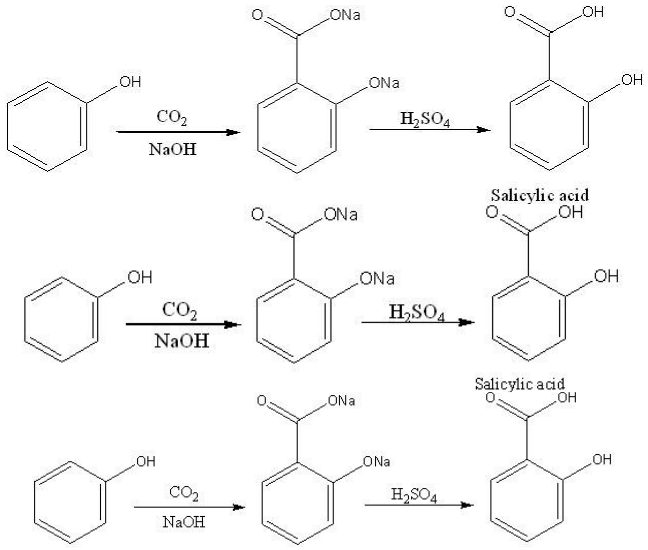
IUPAC NAME: 2-acetoxybenzoic acid
CHEMICAL FORMULAE: $C_9H_8O_4$
PHYSICAL STATE: Colourless, Odorless, white crystalline powder
MELTING POINT: 137^oC (with decomposition)
BOILING POINT: 140^oC
SPECIFIC GRAVITY: 1.35
SOLUBILITY IN WATER: Soluble

Synthesis of Aspirin:

Aspirin is commercially synthesized using a two-step process known as the Kolbe-Schmitt reaction.

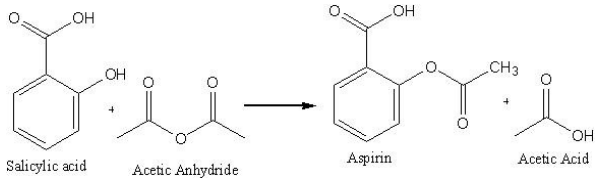
Step 1:

Phenol is treated with sodium hydroxide generating sodium phenoxide, which is then reacted with carbon dioxide under high temperature and pressure to yield sodium salicylate, which is acidifed, yielding salicylic acid.



Step 2:

Salicylic acid is then acetylated using acetic anhydride, yielding aspirin and acetic acid as a byproduct. The yield of this reaction is very low due to the relative difficulty of its extraction from an aqueous state. For bulk production the salicylcate is acidified with phosphoric acid under reflux for 1 hour 40 minutes.

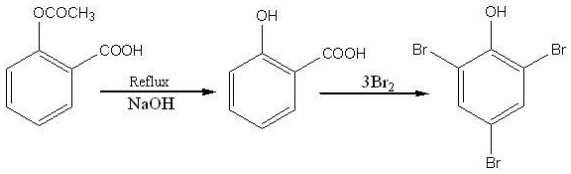


Application and Side Effects of Aspirin:

1. Acetylsalicylic acid is used as analgesic, antipyretic, anticoagulant and anti-rheumatic.
2. It is also used as an additive in food, animal feed, drug and cosmetic.
3. Low doses of aspirin may be given immediately after a heart attack to reduce the risk of another heart attack or death of cardiac tissue.
4. It has been used for the treatment of rheumatoid arthritis, rheumatic fever, and mild infection.
5. Large doses of aspirin cause acid-base imbalance and respiratory disturbances and can be fatal, especially in children.
6. Gastrointestinal ulcers, stomach bleeding, and tinnitus are the main undesirable side effects of aspirin.

Principle and Procedure:

The amount of aspirin can be determined by brominating using **$KBrO_3$-KBr** mixture. A definite amount of aspirin is refluxed with NaOH. Then salicylic acid is formed. The excess brominating mixture formed is titrated with standard thio.



Preparation of KBr-KBrO3 solution:- Dissolve 75 g KBr & 5.36 g of $KBrO_3$ in H_2O and make upto 1litre.

a) Standardisation of $Na_2S_2O_3$:

0.5 g $K_2Cr_2O_7$ is weighed accurately and made upto 100 mL. 20 mL is pipetted out into a conical flask. Then add 3 mL con.HCl followed by 5mL 10% KI and titrated against $Na_2S_2O_3$ using starch as indicator.

b) Estimation of aspirin:

1.5g aspirin is weighed out into an R.B flask. Then 40 mL 10% NaOH is added and refluxed for 15 min. Transfer the solution quantitatively into a 250 mL standard flask, made upto the mark. From that 20 mL is pipette, acidified with 2 mL con. HCl. Then add 50 mL of brominating mixture, shake well for 15min. Then 10 mL 10% KI is added & diluted with H_2O and titrated against standard $Na_2S_2O_3$ using starch as indicator.

Calculation:

Normality of thio = N_1 Weight of aspirin = W g

50 mL brominating mixture = X mL thio

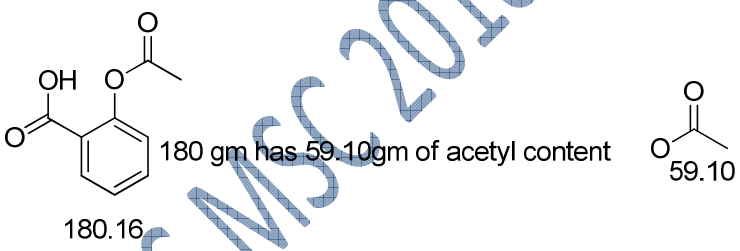
20 mL aspirin + 50 mL brominating mixture = Y mL

Amount of thio = X - Y = Z mL Normality of aspirin = Z x N_1 / 20 = N_2

Amount of aspirin in the whole of the given solution = (N_2 x Equivalent weight of aspirin) / 4 = A g

% of aspirin = (A x 100) / W = B %

Amount of Acetyl Group:



CHAPTER-7:

OBJECTIVE: Determination Of Phenols By Bromination Reagents

Step-1: Potassium bromate-bromide solution, 0-2N. Dissolve 5-567 g. of A.R. potassium bromate and 75 g. of pure potassium bromide in water, and dilute to 1 litre in a volumetric flask. (The large excess over 5 equivalents of potassium bromide serves to ensure the complete reduction of the bromate when the solution is acidified and also to increase the solvent power of the solution for free bromine.)

Sodium thiosulphate solution, 0-2N. Dissolve about 25 g. of A.R. sodium thiosulphate pentahydrate in 1 litre of freshly-boiled and cooled distilled water. Standardise the solution with A.R. potassium iodate.

Iodine solution

It is not difficult to prepare high purity iodine through sublimation, but - due to its volatility - iodine is difficult to weight accurately, as it tends to run away. To minimize losses it should be weight in closed weighing bottle. Iodine should be kept in a closed bottles also because it is highly corrosive and it vapor can damage delicate mechanism of analytical balance. Commonly used solutions are 0.05M (0.1 normal).

To minimalize losses it is important to transfer iodine to the solution as fast as possible, or even to weight a 1% excess. Solution should be kept in dark glass bottle with grinded glass stopper and standardized every few weeks or before use.

Starch indicator solution.

Starch solution is used for end point detection in iodometric titration. To prepare starch indicator solution, add 1 gram of starch (either corn or potato) into 10 mL of distilled water, shake well, and pour into 100 mL of boiling, distilled water. Stir thoroughly and boil for a 1 minute. Leave to cool down. If the precipitate forms, decant the supernatant and use as the indicator solution. To make solution long lasting add a pinch of mercury iodide or salicylic acid, otherwise it can spoil after a few days.

Potassium iodide solution, 20 per cent.

55.97 gm is added to 100 ml volumretric flask capacity to make 20% KI w/v

55.97 gm is added to 80 gm of H2O in volumretric flask to make 20% KI w/w

Preparation of Iodine solution

Iodine 0.1 N: Weigh 40 g of potassium iodide (KI) in a 500 mL glass-stoppered flask and dissolve in 100 mL of purified water. Let the solution come to room temperature, add 12.7 g of resublimed iodine (I2), restopper the flask, and swirl the flask until the iodine is completely dissolved. Transfer the solution quantitatively to a 1 L volumetric flask, add 3 drops of hydrochloric acid (37% HCl; sp g 1.19) and dilute to 1 L with purified water. Mix thoroughly and transfer to a glasss toppered alkali-resistant, amber-colored bottle. Iodine 0.01 N: Dilute 100 mL of 0.1 N iodine to 1 L in a volumetric flask

Standardization of iodine Solution

Iodine 0.1 N: Weigh accurately 0.18-0.22 g arsenious trioxide (As2 O3) (dried 1 hr., 105 °C) National Institute of Science and Technology, U. S. Department of Commerce Sample 83, in a 250 mL Erlenmeyer flask. Dissolve in 10 mL of 1.0 N sodium hydroxide (NaOH) and add 75 mL of purified water. Add 10 mL of 1.0 N hydrochloric acid (HCl) and 20 mL of a saturated sodium bicarbonate (NaHCO3) solution. Add 2 mL of starch indicator and titrate with iodine solution to appearance of the first permanent blue tinge.

Sodium thiosulfate solution

Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) can be realtively easily obtained in a pure form, but it is quite difficult to obtain samples with known amount of water of crystallization, as the exact composition of the nominal pentahydrate is highly temperature and humidity dependent. Thus solution has to be standardized against potassium iodate KIO_3 or potassium dichromate $K_2Cr_2O_7$.

Commonly used solutions are 0.1M (0.1 normal).

PROCEDURE

Weigh out accurately about 0-25 g. of the phenol, dissolve it in 5 ml. of 10 per cent, sodium hydroxide solution, and dilute the solution to 250 ml. in a volumetric flask. Pipette 25 ml. of the phenol solution into a 500 ml. iodine flask, followed by 25 ml. of the bromate-bromide solution, and then dilute with 25 ml. of

water. Add 5 ml. of concentrated hydrochloric acid, and stopper the flask immediately. Shake the flask for 1 minute to mix the reactants, and allow to stand for 30 minutes with occasional

swirling of the contents of the flask. Cool the flask under the tap or in ice water, place 10 ml. of 20 per cent, potassium iodide

solution in the cup around the stopper. Slightly dislodge the stopper whereupon the iodide solution is drawn into the flask with no loss of bromine. Shake the flask well for 30 seconds and allow to stand for 10 minutes (1). Remove the stopper and wash the neck of the flask and the stopper with a little water. Titrate the free iodine, which is equivalent to the excess of bromine taken, with 0- *IN* sodium thiosulphate ; add about 1 ml. of starch solution near the end point. Carry out a blank analysis, using 25 ml. of the bromate-bromide reagent and 25 ml. of water, the procedure being otherwise identical with the analysis proper.

Note.

(1) The precipitate formed with phenol may contain, in addition to tribromophenol, some tribromophenol bromide :

$$C_6H_6OH + 4Br \longrightarrow C_6H_2Br_3.OBr + 4HBr$$

This is of no consequence, as it is converted into tribromophenol when potassium iodide is added to the acid solution :

$$C_6H_2Br_3OBr + 2KI + 2HCl \longrightarrow C_6H_2Br_3.OH + 2KCl + HBr + I_2$$

the extra bromine thus combined reacting as if it were free bromine. It is advisable to allow the solution to stand for 5-10 minutes in the presence of potassium iodide solution to ensure that all the tribromophenol bromide is decomposed.

It may be noted that the simple procedure given above is not applicable to 3-naphthol; the latter (about 0-75 g., accurately weighed) should be dissolved in 10 ml. of 10 per cent, sodium hydroxide solution and diluted to 250 ml. in a volumetric flask. For the titration, use 25 ml. of the P-naphthol solution, 25 ml. of the bromate-bromide solution and 15 ml. of chloroform ; cool in ice for 5 minutes. Add 5 ml. of concentrated hydrochloric acid, stopper the flask, shake gently so that the brominated product dissolves in the chloroform, and cool in an ice bath for a further 5 minutes. Add 10 ml. of 20 per cent, potassium iodide solution, allow to stand for 10 minutes, and titrate with 0- *IN* sodium thiosulphate solution.

Shake vigorously before the end point is reached as the chloroform tends to retain the last traces of iodine rather tenaciously. Perform a blank titration under the same conditions and thus compensate for the slight attack on the chloroform by the bromine.

CALCULATION

Calculate the percentage purity of the phenol from the expression:

$$\% \text{ Purity} = \frac{(V1-V2)\times N\times M\times 100}{W\times 2000\times Z}$$

where

V_1= volume of sodium thiosulphate solution used in the analysis ;

V_2 = volume of sodium thiosulphate solution used in blank ;

N = normality of sodium thiosulphate solution ;

M =molecular weight of Phenol (xx.xx) ; and

W = weight (g.) of sample.

Z=number of bromine atoms substituted in the phenol.

Alternatively,

the blank analysis may be omitted and the percentage purity of the phenol calculated from formula (2). The student is recommended to perform the blank titration and to calculate the result by both methods.

$$\% \text{ Purity} = \frac{(VN2-V2N1)\times 100\times M}{W\times 2000\times Z}$$

where

V = volume (ml.) of bromate solution used for the titration ;

$N2$ = normality of bromate solution ;

V2 = volume (ml.) of sodium thiosulphate solution used ;

$N1$ = normality of sodium thiosulphate solution ;

M =molecular weight of Phenol (xx• xx) ; and

W = weight (g.) of sample.

Z=number of bromine atoms substituted in the phenol.

$$KBrO_3 + 5KBr + 6HCl \longrightarrow 6KCl + 3H_2O + 3Br_2$$

$$C_6H_5OH + 3Br_2 \longrightarrow Br_3C_6H_2OH + 3HBr$$

The volume of N bromate solution reacting with W g. of the sample is $(VN_2 - V_2N_1)$ ml.

$\therefore$ Weight of bromine used by W g. of sample $= (VN_2 - V_2N_1) \times 80/1000$ g.

$\therefore$ Weight of bromine used by M g. of sample $= \dfrac{(VN_2 - V_2N_1) \times 80 \times M}{W \times 1000}$ g.

But 1 mol of pure phenol would react with 3 mols of bromine or 6×80 g. of bromine,

$$\therefore \quad \% \text{ Purity} = \frac{(VN_2 - V_2N_1) \times 80 \times M \times 100}{W \times 1000 \times 6 \times 80}$$

$$= \frac{(VN_2 - V_2N_1) \times M \times 100}{W \times 2000 \times 3}$$

For a phenol which reacts with Z mols of bromine :

$$\% \text{ Purity} = \frac{(VN_2 - V_2N_1) \times M \times 100}{W \times 2000 \times Z} \qquad (2)$$

Equation (2) may be written in the form :

$$\% \text{ Purity} = \left(\frac{VN_2}{N_1} - V_2\right) \times \frac{N_1 \times M \times 100}{W \times 2000 \times Z}$$

This is identical with equation (1), VN_2/N_1 representing the theoretical value of the blank.

References:

1. **Vogel , practical organic chemistry**
2. **Mann, practical organic chemistry**