गुरु घासीदास विश्वविद्यालय, बिलासपुर
Guru Ghasidas Vishwavidyalaya, Bilaspur
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Practical Lab Manual

LAB MANUAL ORGANIC CHEMISTRY

(M.Sc. IIIrd Semester)

Subject Code: CYUALB3

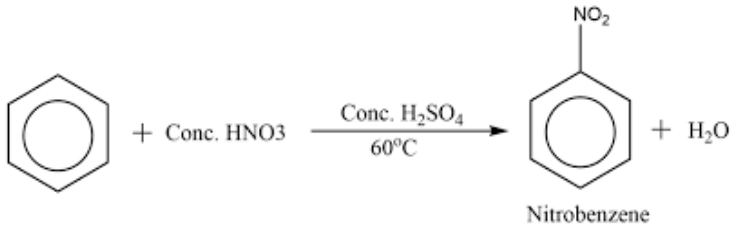
Course Instrctor: Dr. Sunil Kumar Singh

Contents

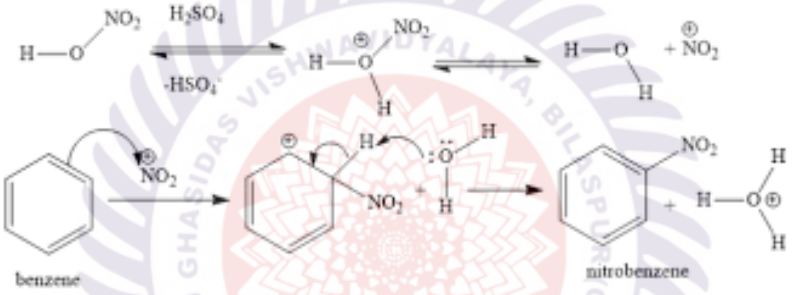
Two-step synthesis of m-dinitrobenzene
Benzene → Nitrobenzene → m-dinitrobenzene

1. Synthesis of Nitrobenzene from benzene

Nitrobenzene is obtained by the nitration of benzene with a mixture of conc. Nitric acid and conc. Sulphuric acid.



Benzene reacts very slowly with conc. Nitric acid to produce nitrobenzene. The reaction rate is increased many folds by using a mixture of conc. Nitric acid and conc. Sulphuric acid. Sulphuric acid acting as a very strong acid, protonates nitric acid and produces nitronium ion as per the following reaction.



Nitration follows the general mechanism of an electrophilic aromatic substitution.

Chemicals Required :

1. Benzene 5ml
2. Conc. Nitric acid 7ml
3. Conc. Sulphuric acid 7ml

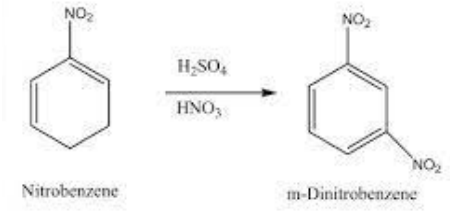
Procedure :

Add conc. sulphuric acid (7 ml) in small portions to cone, nitric acid (7 ml) in a dry conical flask. Cool the flask in and bot Add the cooled mixture drop-wise through acid (ml) in a dry conical flask Cool uken in a round bottomed flask (100 ml capacity). Shake the flask separating fynel to be cool under a tap so that the temperature does not rise above 50 When the additing the add heat the reaction mixture on a water bath at 50-60° for about 20 min. Shake the flask from time to time (to check whether the nitration is complete Add a drop of the reacter the flask from time ima test tube. In case the drop sinks to the bottom, the reaction is considered complete). Cool the reaction mixture and pour into cold water (about 90-100 ml) in a beaker. Stir the mixture and then allow to settle. Decant the upper aqueous layer and transfer the remaining lower layer to a separatory funnel Wash it with sodium hydroxide solution. Dry the remaining crude nitrobenzene over calcium chloride (1-2 g) and distil. Collect the fraction boiling between 210-211.

Yield: 4 g.

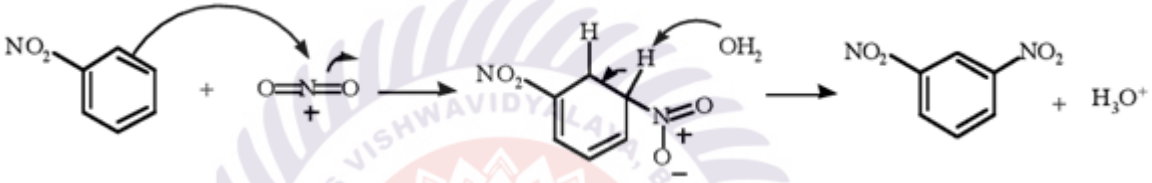
2. Synthesis of m-Dinitrobenzene from Nitrobenzene

Nitration of nitrobenzene with a mixture of fuming nitric acid and conc. Nitric acid and conc. Sulphuric acid gives m-Dinitrobenzene.



Synthesis of m-Dinitrobenzene from Nitrobenzene

The -NO_2 group in nitrobenzene is an electron-withdrawing or a deactivating and meta-directing group due to the following resonances structures of nitrobenzene. Due to the electron-withdrawing effect of -NO_2, the o- and p-position acquire a low electron density compared to the meta position and hence the nitration occurs at this position as follows :



Synthesis of m-Dinitrobenzene from Nitrobenzene

Chemicals :

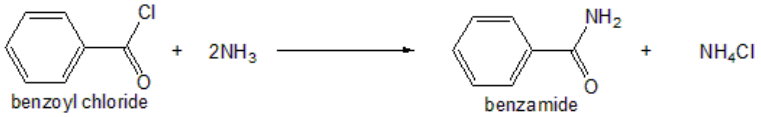
1. Nitrobenzene 1ml
2. Fuming nitric acid 3ml
3. Conc. Sulphuric acid 4ml

Procedure :

Add nitrobenzene (1 ml) in small lots to the nitrating mixture (prepared by adding carefully 4 mi sulphuric acid to 3 ml fuming nitric acid) in a round bottomed flask fitted with a water condensor S the reaction frequently during the addition. Heat on a wire gauze for 15 min. (or till the reaction complete) with occasional shaking. Cool the reaction mixture and pour carefully into ice cold water (30 in a beaker. Filter the separated m-dinitrobenzene and wash with cold water (till free of acid). Crystall from alcohol.
Yield: 1.1g.
M.P.: 89-90°C.

Two-step synthesis of Benzoic acid
Benzoyl chloride → Benzamide → Benzoic acid

1. Synthesis of Benzamide from Benzoyl chloride



Benzamide from Benzoyl chloride

Chemicals Required :

1. Conc. Ammonia 5ml
2. Benzoyl chloride 2ml
3. Hot distilled water

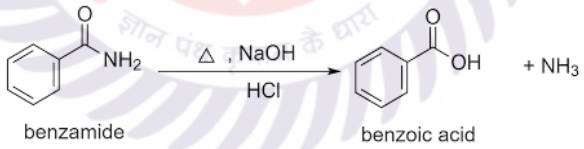
Procedure :

1. In a conical flask, take a mixture of conc. Ammonia and 5ml water.
2. Add 2ml of benzoyl chloride, cork the flask and shake vigorously.
3. Hold the cork securely during shaking since heat generated during the reaction.
4. After 15min not even a trace of benzoyl chloride remains.
5. Filter the fine flakes, wash with cold water and recrystallize from hot water.

Yield = 1.5 gm
MP = 130°C

2. Synthesis of Benzoic acid from Benzamide :

Benzamide on hydrolysis with alkali gives the salt of benzoic acid and ammonia.



Benzoic acid from benzamide

Chemicals Required :

1. Benzamide 2.0g
2. Sodium hydroxide solution 15ml, 10%

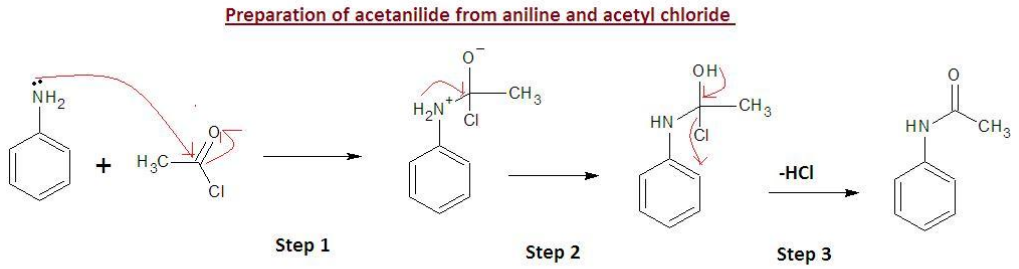
Procedure :

Reflux a mixture of benzamide (2 g) and sodium hydroxide solution (10%, 15 ml) in a round bottomed flask fitted with a water condenser on a sand bath (95-100°) for 30 minutes till there is no more evolution of ammonia. (This can be inferred either by the disappearance of ammonical odour or by bringing a glassrod dipped in conc. HCl to the mouth of the condenser, there are no dense white fumes of NH_4 Cool the resultant solution in an ice bath and acidify with dil. hydrochloric acid. Filter separated benzoic acid. Recrystallise from water.

Yield : 1.2 g
M.P. : 122°C

Two step synthesis of p-nitroacetanilide
Aniline → Acetanilide → p-nitroacetanilide

1. **Synthesis of acetanilide from aniline :**
   It is prepared by acetylation of aniline with acetyl chloride or acetic anhydride and glacial acetic acid.



Chemicals Required :

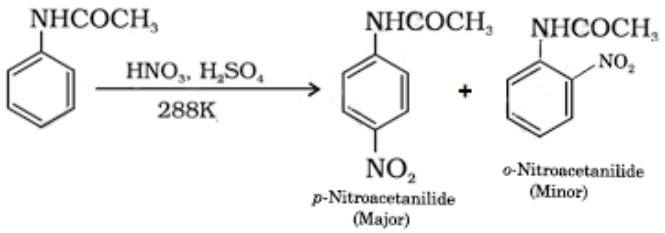
1. Aniline
2. Acetyl chloride
3. Glacial acetic acid
4. Ice cold water

Procedure :

1. Take 1ml of aniline in a beaker and to it add 1ml of glacial acetic acid with constant stirring.
2. Add 1ml of acetyl chloride to the above solution dropwise by keeping the beaker in ice-cold water.
3. Heat the solution on boiling water bath for 5min.
4. Cool the solution. After it has reached room temperature add 10ml of ice-cold water with constant stirring.
5. Filter it and dissolve the obtained brown solid into 10-20ml of distilled water and heat it on a water bath for 5min.
6. Again filter this solution containing unreacted aniline.
7. Leave the obtained filtrate to cool at room temperature.
8. Filter the white coloured crystals of acetanilide and dry in sunlight.

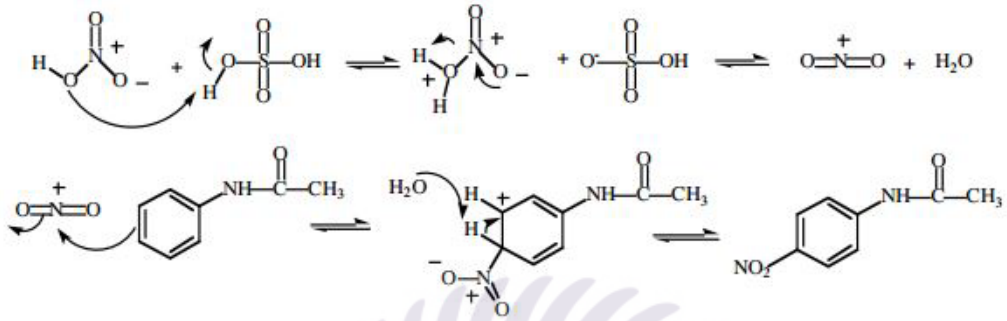
   Yield : 1.48 gm
   M.P. : 114^{o}C

2. **Synthesis of p-Nitroacetanilide from acetanilide :**
   Nitration of acetanilide with a mixture of conc. Sulphuric acid and conc. Nitric acid yields p-nitroacetanilide and a very small amount of o-nitroacetanilide.



Synthesis of p-Nitroacetanilide from Acetanilide

Mechanism



Chemicals Required :

1. Acetanilide 2gm
2. Conc. Nitric acid 0.9ml
3. Conc. Sulphuric acid 4.6ml
4. Glacial acetic acid 2.5ml

Procedure :

Add conc sulphuric acid (3.9 ml) to a well stirred solution of acetanilide (2 g) in glacial acetic acid (2.5 ml) Cool the resultant solution in an ice bath, and add drop-wise a mixture of conc nitric acid and conc sulphuric acid to it (0.9 ml conc. HNO3 +0.7 ml conc. H_2SO_4). Maintain the temperature of the reaction mixture below 10°C. Allow the reaction mixture to stand for 30-40 minutes at room temperature and then add crushed ice (-20 g) with surring. Filter the separated p-nitroacetanilide, wash with water and recrystallise from alcohol.

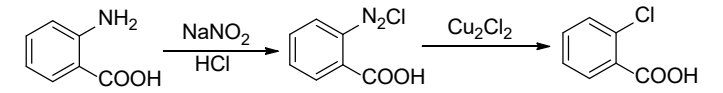
Yield: 1.2 gm

M.P. : 214-215°C.

Synthesis of *o*-chlorobenzoic acid from anthranilic acid via Sandmayer reaction

Aim : To prepare *o*-chlorobenzoic acid from anthranilic acid.

Theory : Whenever a diazonium salt solution is treated with cuprous halide solution in presence of the corresponding halogen acid the diazo group is replaced by a halogen atom : the reaction being known as Sandmayer reaction.



Anthranilic acid o-chlorobenzoic acid

Chemicals required :

1. Anthranilic acid = 7gm
2. Concentrated HCl = 50ml
3. Sodium nitrite = 3.5gm
4. Copper sulphate = 13gm
5. Sodium chloride = 6gm
6. Copper turnings = 7gm
7. Ice

Procedure :

1. Dissolve 7gm of the anthranilic acid in 60ml dil HCl (10 ml conc. HCl diluted with 50ml water).
2. Cool it to about 5°C.
3. Add slowly to it ice cold solution of 3.5gm of sodium nitrite in 12.5ml of water until a slight excess of nitrous acid is present.
4. Side by side prepare cuprous chloride solution in the following way : Dissolve 13gm of copper sulphate and 6gm of sodium chloride in 25ml water in round bottomed flask.
5. Heat this solution to boiling, add about 40ml concentrated hydrochloric acid and 7gm of copper turnings.
6. Reflux the solution until it becomes colourless.
7. Cool this solution in ice.
8. Now to this ice cold solution add the ice cold diazonium solution slowly and with shaking.
9. Allow the reaction mixture to stand for about 2-3 hours with frequent shaking.
10. Filter of the product, wash with a little cold water and then recrystallize from hot water containing a little alcohol and a little decolourising carbon.

Yield : 7gm
M.P. : 138 – 139 °C

Separation of Dyes using thin layer chromatography

Aim : To prepare the TLC plate and separation of dyes using thin layer chromatography technique.

Apparatus required : Glass slide, beaker, china dish, glass rod, test-tube, capillary tube etc.

Chemical required : Dyes, silica gel G, solvent (Ethyl actetate : Hexane, 25:75), acetic acid, Distilled water.

Principle : TLC principle works on a solubility rule "Like dissolves like" and is followed for separation of mixture of polar, non-polar, mild polar compound from the extracts on a static phase (silica gel) and moveable phase such as ethyl acetate, chloroform etc. that runs on static phase. These mobile phases can is combination of different solvents.

The mobile phase is drawn up through the stationary phase by capillary action, few compound in mixture would dissolve in mobile phase that goes up the plate. Some compounds will remain on stationary phase. The movement of compound from the mixture depend on the physical properties, molecular structure and functional group. The amount that each component of a mixture travel can be quantified using retention factor.

$$\textbf{Retention factor} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Rf value will always be less than 1, as the Rf value increases, the retention of solute decreases.

Procedure :

1. Preparation of TLC plate :
   a. Adsorbents used are generally alumina or silica gel containing a little calcium sulphate to increase the strength of the coating.
   b. Mix the Silica gel with water and acetic acid by using glass rod and prepare a slurry.
   c. Spread this slurry as a thin layer on a clean glass slide and then dry it.
   d. Activate it by heating in oven for 30min at 110 ^{o}C.
   e. Thickness of the silica layer should be around 100-250 µm.

2. Separation of dyes :
   a. With a pencil, make a thin mark at the bottom of the plate to apply sample spot.
   b. Draw the spot on the line drawn one of each dye and one in the middle of their mixture.
   c. Pour the mobile phase into the beaker.

d. Now put the prepared plate with spotting inside the beaker in such a manner that the spot should lie above the level of solvent.
e. Leave the aromatic chamber undisturbed for some time.
f. The solvent spread the different pigments of the mixture to various distance.
g. Remove the glass slide and allow to dry.
h. Calculate the retention factor and identify the spots in the mixture.

Thin layered chromatographic separations of Glucose, Fructose and Sucrose.

Object : To separate Glucose and Fructose from the given mixture by using thin layer chromatography.

Principle :

It is an adsorption type of chromatography and is used for qualitative determination. Silica gel and alumina are most commonly used as an adsorbent/ stationary phase. The moving/mobile phase may be a pure solvent or a mixture of solvents. Components present in the solutions are separated on the basis of their polarity.

Chemicals Required :

1. Glass plate
2. Beaker
3. Silica gel G
4. Ethyl acetate
5. Dil. H_2SO_4
6. Capillary tube
7. Distilled water.

Procedure :

1. Prepare a slurry of silica gel and apply it on glass plate.
2. Let the plate dry for half an hour or until the silica dries.
3. Draw 2 lines from each end at the distance of 2cm.
4. Spot the given solution on the stationary phase with the help of a capillary tube.
5. In a beaker containing ethyl acetate, dip the end of glass plate where the spotting has been done such that the spot does come in contact with the mobile phase.
6. Leave it for some time so that the solvent runs till the line drawn in the opposite end.
7. After that take it out of the beaker and spray with dil. H2SO4.
8. Heat the plate on a hot plate at 70-80^{o}C for half an hour.
9. The black spots of Glucose and Fructose can be seen on the plate.
10. Measure their distance from the lower line and calculate their Rf value.

COLUMN CHROMATOGRAPHIC SEPARATION OF PIGMENTS FROM GREEN LEAVES

Object : Column chromatographic separation of pigments from green leaves.

Principle : The success of a separation by column chromatography depends on the choice of the stationary and mobile phases. The stationary phase material is filled in a column. Any of the three possible mechanisms: partition, adsorption or ion exchange can be employed by the use of a particular type of the stationary phase inside the column. For example, for the separation based on adsorption a n adsorbent is packed in the column.

The choice of the mobile phase depends on the nature of the substance and how strongly it is adsorbed. In a number of cases such as alumina and silica gel as the adsorbent, the mobile phase is generally a non-polar solvent such as petrol and benzene because polar groups such as hydroxyl-(OH) group in water and in ethanol would cause desorption. Eluents containing two or more solvents may be used for better results. In such cases the polarity is increased by adding a polar solvent with a non-polar one.

Requirements :

1. Chromatographic column
2. Beaker
3. Conical flask
4. Wash bottle
5. Calcium carbonate
6. Benzene
7. Petroleum ether
8. Ethyl alcohol

PROCEDURE :

1. **Preparation of the Extract:** Take 5-10 g of fresh grass (or leaves of a green plant) cut it up into fine pieces in a mortar, grind for about 30 seconds, add 10 ml of ethyl alcohol and 20 ml of petroleum ether, grind again. Decant the liquid into a separatory funnel after filtering through glass wool placed in an ordinary funnel. Add 10 ml alcohol and 20 ml petroleum ether again to the mortar containing grass, grind, and transfer the liquid after decantation to the separatory funnel containing the first fraction. Shake gently. A light green emulsion may form if shaken vigorously. Allow to settle the layers. The bottom layer is water-ethanol layer and the upper layer of petroleum-ether contains grass extract. Remove the bottom layer and wash the petroleum layer with water for 3 or 4 times until the layer is clear. Remove the aqueous layer.

   The extract is now free from alcohol but contains water in very small amounts. Transfer the upper layer containing the extract to a dry conical flask. To this, add anhydrous sodium sulphate (dried by heating in an oven hot plate before use), shake the flask and leave it over for about 15 minutes to remove any water present with the extract. Transfer the extract to a clean and dry test tube, cover it and take it for chromatography.
2. **Preparation of column:** Take a glass column or a burette of about 20cm in length and 7-8 mm diameter tube. Place a small wad of cotton wool as the column support. Pack the column with anhydrous calcium carbonate (dried by heating in a china dish over a burner), tap it regularly with a glass rod. Add the adsorbent in small portions and gently press down until a column of 8-

10 cm has been uniformly packed. Place a small wad of cotton wool at the top of the calcium carbonate column and use it for chromatography.

3. Take the uniformly packed column containing calcium carbonate and fix it in a stand vertically.
4. Take 1-2 ml of dried extract of leaves, drip into the column in the form of a thin layer of solution, and allow to run evenly into the adsorbent until: a green zone 3-4 mm deep is formed at the top of the column. This is known as the loading of the sample.
5. Add the developer (benzene) to the column and allow the developer through the column packing till separate bands are observed.
6. Note the colour of different bands and their order.

RESULT AND DISCUSSION :

The bands observed on the column are of different colours. The uppermost thin yellowish-green zone is chlorophyll-b, below this is the bluish-green zone of chlorophyll-a, the next orange-yellow zone contains xanthopylls and the lowest orange zone contains carotenes. The carotenes are least adsorbed by the adsorbent and can be easily washed out of the column.

Three main interactions are to be considered in column chromatography: the activity of the adsorbent, the polar behaviour of the substance and the polarity of the eluting solvent. This experiment is based on the results of the inventor of the technique of chromatography, M. Tswett, who applied the technique to separate various plant pigments using calcium carbonate as the stationary phase packed in a column.

References:

1. College Practical Chemistry, VK Ahluwalia, S Dhingra, A Gulati, Universities Press.
2. Advanced Practical Organic Chemistry, OP Agrawal, Krishna Publication.