

**LAB MANUAL**  
**M. Sc. (Previous)**



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## Qualitative Analysis of Organic Compounds

The analysis and identification of unknown organic compounds constitutes a very important aspect of experimental organic chemistry. There is no definite set procedure that can be applied overall to organic qualitative analysis. A systematic approach based on the scheme given below will give satisfactory results. Qualitative tests that require substantial quantities of a number of (often hazardous) chemicals to be stocked in the lab for experimental use.

### General Scheme of Analysis

#### A. Preliminary Test

Note physical characteristics: solid, liquid, color, and odor. Compounds that are yellow to red in color are often highly conjugated. Amines often have a fish-like odor, while esters usually have a pleasant fruity or floral smell. Acids have a sharp, biting odor. Odors can illicit information about your unknown; it is wise to sniff them with caution. Some compounds can have corrosive vapors or make you feel nauseous.

#### B. Physical Constants

Determine the boiling point or melting point. Distillation is recommended in case of liquids. It serves the dual purpose of determining the boiling point as well as purifying the liquid for subsequent tests.

#### C. Solubility Tests

The solubility of the unknown in the following reagents provides very useful information. In general, about 1 mL of the solvent is used with approximately 0.1 g or 0.2 mL (2-3 drops) of the unknown compound. Assistance in analyzing the results from your solubility tests can be found in the solubility flowchart given in Fieser and Williamson on page 606. (Careful, this flowchart is much more complex than our lab requires and often can make it more difficult to form conclusions from your solubility data.)

### Procedure for Determining Solubility of Organic Compounds

The amounts of material to use for a solubility test are somewhat flexible. Use 2-3 drops of a liquid or approximately 10 mg of a solid. Unless the solid is already a fine powder, crush a small amount of the solid on a watch glass with the back of a spatula. Do not weigh the solid; simply use enough to cover the tip of a small spatula. Your instructor will demonstrate how to estimate the correct amount. Place the appropriate amount of either your solid or liquid unknown in a small test tube and proceed with the following solubility tests.

#### 1) Water Solubility

Add approximately 1 mL of water to the test tube containing your organic compound. Shake the tube and/or stir with a glass stirring rod. A soluble organic compound will form a homogeneous solution with water, while an insoluble organic compound will remain as a separate phase. You may add additional water, up to 1.5 mL, if your compound does not completely dissolve with the smaller amount.

Check the pH of the water to determine if your unknown is partially or completely soluble in water and whether your compound has changed the pH of the water.

- Litmus turns red: acidic compound
- Litmus turns blue: basic compound
- Litmus neutral: either water soluble general compound or insoluble compound

An organic compound which is soluble in water is typically a low molecular weight polar compound of up to 5-6 carbon atoms or less.

### **Acid-Base Solubility Tests**

[Please write a general chemical reaction(s) for any positive solubility test result(s) that you obtain for your unknown compound. Your reaction should demonstrate how any organic compound with a specific functional group can dissolve/react in an aqueous solution.]

#### **2) 5% NaOH Solubility**

Add approximately 1 mL of 5% NaOH in small portions to the test tube containing your organic compound. Shake the test tube vigorously after the addition of each portion of the aqueous solution. Solubility will be indicated by the formation of a homogeneous solution, a color change, or the evolution of gas or heat. If soluble, then your organic compound is behaving as an organic acid. The most common organic acids are carboxylic acids and phenols. Carboxylic acids are usually considered stronger acids than phenols, but both of these acids should react with 5% NaOH (a dilute strong base).

#### **3) 5% NaHCO<sub>3</sub> Solubility**

Add approximately 1 mL of 5% NaHCO<sub>3</sub> in small portions to the test tube containing your organic compound. Shake the test tube vigorously after the addition of each portion of the aqueous solution. Solubility will be indicated by the formation of a homogeneous solution, a color change, or the evolution of gas or heat. If soluble, then it is behaving as a strong organic acid. If not, then it is a weak organic acid, if it dissolves in NaOH. The most common weak organic acid are phenols. Typically, only a carboxylic acid will react with NaHCO<sub>3</sub>.

#### **4) 5% HCl Solubility**

Add approximately 1 mL of 5% HCl in small portions to the test tube containing your organic compound. Shake the test tube vigorously after the addition of each portion of the aqueous solution. Solubility will be indicated by the formation of a homogeneous solution, a color change, or the evolution of gas or heat. If your compound is HCl-soluble, then it is an organic base. Amines are the most common organic base. If insoluble in all solutions, then your unknown is a large (>5-6 carbon atoms) neutral compound that has none of the acidic or basic organic functional groups mentioned above.

### **SOLUBILITY TABLE**

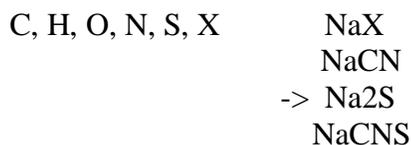
REAGENT AND TEST	CLASS	GROUP OF COMPOUNDS
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Soluble in cold or hot water. (If the unknown is soluble do NOT perform solubility tests below)	Neutral, acidic or basic. (Test with litmus or universal indicator paper)	Lower members of series. Neutral, e.g. alcohols; Acidic, e.g. acids, phenols; Basic, e.g. amines
Soluble in dil. HCl	Basic	Most amines (except III amines with <u>only aromatic groups</u> )
Soluble in dil. NaOH	Acidic	Most acids, most phenols.
Soluble in NaHCO <sub>3</sub>	Strongly acidic	Most carboxylic acids.
Insoluble in water, acid and alkali	Neutral	Hydrocarbons, nitrohydro-carbons, alkyl or aryl halides, esters and ethers. Higher molecular weight alcohols, aldehydes and ketones

### Qualitative Analysis for Elements (for reference only)

In organic compounds the elements commonly occurring along with carbon and hydrogen, are oxygen, nitrogen, sulphur, chlorine, bromine and iodine. The detection of these elements depends upon converting them to water-soluble ionic compounds and the application of specific tests.

#### Lassaigne's Sodium Fusion Test



#### PROCEDURE

Place a piece of clean sodium metal, about the size of a pea into a fusion tube. Add a little of the compound (50 mg or 2 - 3 drops). \* Heat the tube gently at first, allowing any distillate formed to drop back onto the molten sodium. When charring begins, heat the bottom of the tube to dull redness for about three minutes and finally plunge the tube, while still hot, into a clean dish containing cold distilled water (6 mL) and cover immediately with a clean wire gauze.

#### 1. NITROGEN

To a portion (2 mL) of the 'fusion' filtrate add 0.2 g of powdered ferrous sulphate crystals. Boil the mixture for a half a minute, cool and acidify by adding dilute sulphuric acid dropwise. Formation of a bluish-green precipitate (Prussian blue) or a blue solution indicates that the original substance contains nitrogen. If no precipitate appears, allow to stand for 15 minutes, filter and inspect filter paper.

#### 2. SULPHUR (SULPHIDE)

To the cold 'fusion' filtrate (1 mL) add a few drops of cold, freshly prepared, dilute solution of sodium nitroprusside. The latter may be prepared by adding a small crystal of the solid to 2 mL of water. Production of a rich purple colour indicates that the original substance contains sulphur. This test is very sensitive. Only strong positive results are significant.

### 3. HALOGENS (HALIDES)

Acidify a portion (1 mL) of the 'fusion' filtrate with 2N nitric acid, and if nitrogen and/or sulphur are present, boil for 1 - 2 minutes.\* Cool and add aqueous silver nitrate (1 mL), compare with a blank. Formation of a heavy, white or yellow precipitate of silver halide indicates halogen. If a positive result is obtained: acidify the remaining portion of the 'fusion' filtrate with dilute sulphuric acid, boil and cool. Add carbon tetrachloride (1 mL) and a few drops of freshly prepared chlorine water. Shake the mixture.

(a) If the carbon tetrachloride layer remains colourless - indicates chlorine.

(b) If the carbon tetrachloride layer is brown - indicates bromine.

(c) If the carbon tetrachloride layer is violet - indicates iodine.

\*If nitrogen and/or sulphur are also present, the addition of silver nitrate to the acidified 'fusion' solution will precipitate silver cyanide and/or silver sulphide in addition to the silver halides. The removal of hydrogen cyanide and/or hydrogen sulphide is effected by boiling the 'fusion' solution.

#### D. Group Classification Tests

After analysis of the previous tests and the compound's IR spectrum, if needed, further information can be deduced by performing carefully selected functional group classification tests.

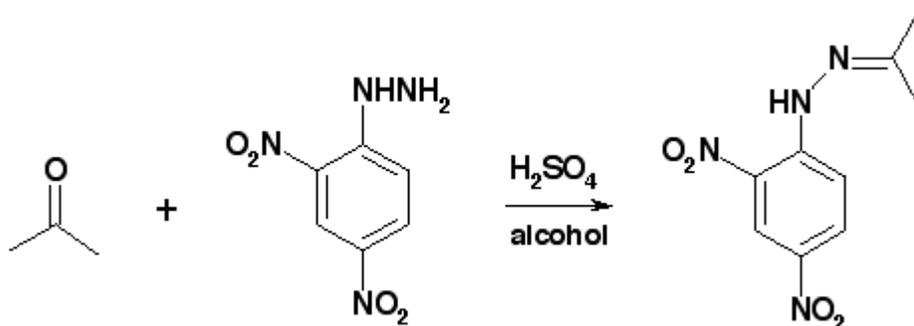
#### Ignition Test for Aromaticity

Place a small amount of compound on the end of a spatula or on a porcelain lid and apply the flame from a Bunsen burner. Highly unsaturated compounds such as aromatic compounds burn with a yellow, sooty flame. Use naphthalene for the solid unknown and toluene or xylene for the liquid known. This test should be done in a hood.

#### Tests for Aldehydes and Ketones

- 2,4-DNP Test for Aldehydes and Ketones

##### Aldehyde or Ketone



#### Procedure

Add a solution of 1 or 2 drops or 30 mg of unknown in 2 mL of 95% ethanol to 3 mL of 2,4-

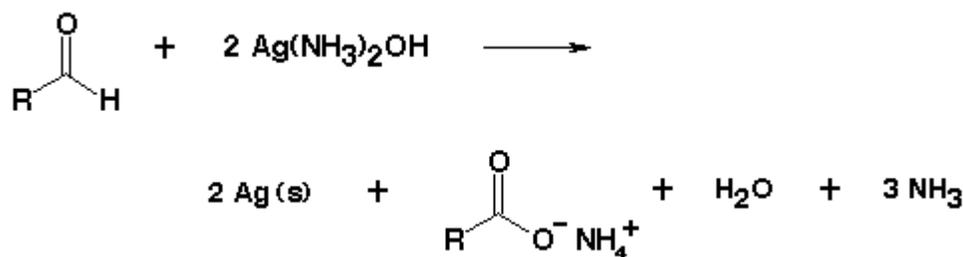
dinitrophenylhydrazine reagent. Shake vigorously, and, if no precipitate forms immediately, allow the solution to stand for 15 minutes. The 2,4-dinitrophenylhydrazine reagent will already be prepared for you.

### Positive test

Formation of a precipitate is a positive test.

### Tollen's Test for Aldehydes

#### Aldehyde



#### Procedure

Add one drop or a few crystals of unknown to 1 mL of the freshly prepared Tollens reagent. Gentle heating can be employed if no reaction is immediately observed.

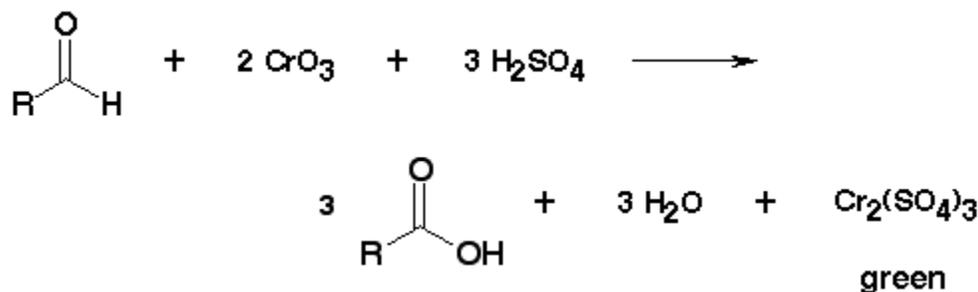
**Tollen's reagent:** Into a test tube which has been cleaned with 3M sodium hydroxide, place 2 mL of 0.2 M silver nitrate solution, and add a drop of 3M sodium hydroxide. Add 2.8% ammonia solution, drop by drop, with constant shaking, until almost all of the precipitate of silver oxide dissolves. Don't use more than 3 mL of ammonia. Then dilute the entire solution to a final volume of 10 mL with water.

#### Positive Test

Formation of silver mirror or a black precipitate is a positive test.

### Jones (Chromic Acid) Oxidation Test for Aldehydes

#### Aldehydes



#### Preparation of Jones' reagent

**Jones reagent** is prepared by dissolving 26.72 grams of chromium trioxide ( $\text{CrO}_3$ ) in 23ml of concentrated sulfuric acid, and then diluting the mixture to 100ml with water.

### Procedure

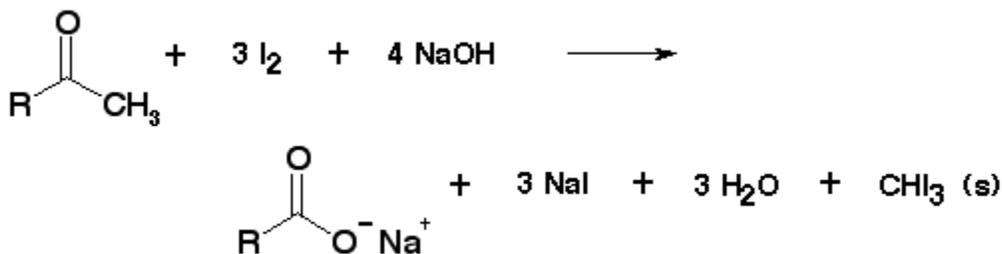
Dissolve 10 mg or 2 drops of the unknown in 1 mL of pure acetone in a test tube and add to the solution 1 small drop of Jones reagent (chromic acid in sulfuric acid). A positive test is marked by the formation of a green color within 5 seconds upon addition of the orange-yellow reagent to a primary or secondary alcohol. Aldehydes also give a positive test, but tertiary alcohols do not. The Jones reagent will already be prepared for you.

### Positive Test

A positive test for aldehydes and primary or secondary alcohols consists in the production of an opaque suspension with a green to blue color. Tertiary alcohols give no visible reaction within 2 seconds, the solution remaining orange in color. Disregard any changes after 15 seconds.

### Iodoform Test for Methyl Ketones

#### Ketone



### Procedure

If the substance to be tested is water soluble, dissolve 4 drops of a liquid or an estimated 50 mg of a solid in 2 mL of water in a large test tube. Add 2 mL of 3 M sodium hydroxide and then slowly add 3 mL of the iodine solution. Stopper the test tube and shake vigorously. A positive test will result in the brown color of the reagent disappearing and the yellow iodoform solid precipitating out of solution. If the substance to be tested is insoluble in water, dissolve it in 2 mL of 1,2-dimethoxyethane, proceed as above, and at the end dilute with 10 mL of water.

### Preparation of reagent

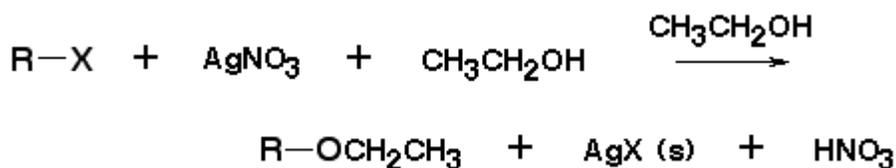
The iodine-potassium iodide solution is prepared from 10 g of iodine and 20 g of potassium iodide in 100 mL of water.

### Positive Test

Formation of solid iodoform (yellow) is a positive test. (Iodoform can be recognized by its odor and yellow color and, more securely, from the melting point 119°-123°C).

### Tests for Halides

#### Silver Nitrate in Ethanol Test



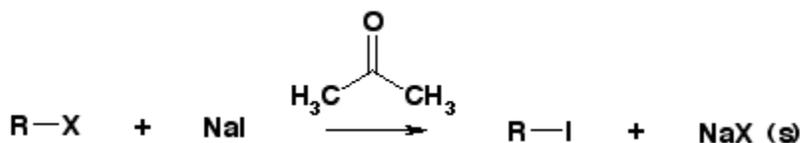
**Standards**, as done in the Classification Tests for Halides lab

1-chlorobutane	1-bromobutane	1-iodobutane
2-chlorobutane	2-bromobutane	2-iodobutane
2-chloro-2-methylpropane	2-bromo-2-methylpropane	
benzyl chloride	bromobenzene	

### Procedure

Place approximately 0.25 mL of each compound into a test tube. Add 2 mL of a 1% ethanolic silver nitrate solution to the material in each test tube, noting the time of addition. After the addition, shake the test tube well to ensure adequate mixing of the compound and the solution. Record the time required for any precipitates to form. If no precipitates are seen after 5 minutes, heat the solution on the steam bath for approximately 5 minutes. Note whether a precipitate forms in the test tube. Continue slow reactions for up to 45 minutes at room temperature.

### Sodium Iodide in Acetone Test



### Standards

Reference tests done in Classification Tests for Halides Lab

### Procedure

In a test tube place 0.25 mL or 0.2 g of your unknown. Add 2 mL of a 15% solution of sodium iodide in acetone, noting the time of addition. After the addition, shake the test tube well to ensure adequate mixing of the unknown and the solution. Record the time needed for any precipitate to form. After about 5 minutes, if no precipitate forms, place the test tube in a 50°C water bath. Be careful not to allow the temperature of the water bath to go above this temperature since the acetone will evaporate, giving a false positive result. After 6 minutes more in the bath, if no precipitates are visible, remove the test tube and let it cool to room temperature. Note any change that might indicate that a reaction has occurred. Continue slow reactions for up to 45 minutes at room temperature.

### Unsaturation



### Procedure

Dissolve 1 drop or 0.02 grams of the unknown in 0.5 mL reagent grade acetone. Add a 1% aqueous solution of potassium permanganate dropwise with shaking. If more than one drop of reagent is required to give a purple color to the solution, unsaturation or an easily oxidized functional group is present. Run parallel tests on pure acetone and, as usual, the standards listed above.

### Positive Test

The disappearance of the  $\text{KMnO}_4$ 's purple color and the appearance of a brown suspension of  $\text{MnO}_2$  is a positive test.

### Ignition Test for High Degrees of Unsaturation

#### Procedure

Heat a small sample on a spatula. First, hold the sample near the side of a bunsen burner to see if it melts normally and then burns. Heat it in the flame. Aromatic compounds often burn with a smoky flame.

#### Positive Test

A sooty yellow flame is an indication of an aromatic ring or other centers of unsaturation.

### Tests for Carboxylic Acids

#### pH of an Aqueous Solution for Carboxylic Acids

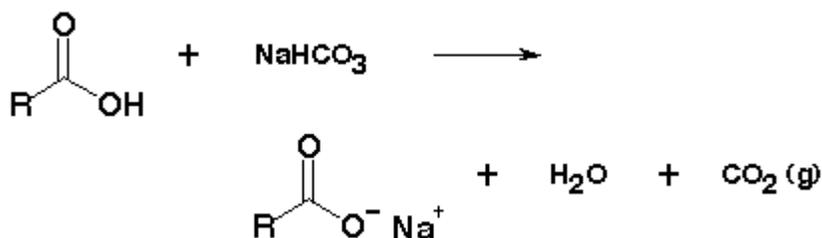
#### Standard

5% Acetic acid solution

#### Procedure

See procedure for solubility tests with water.

#### Sodium Bicarbonate Test for Carboxylic Acids



#### Standard

5% Acetic acid solution

#### Procedure

A few drops or a few crystals of the unknown sample are dissolved in 1 mL of methanol and slowly added to 1 mL of a saturated solution of sodium bicarbonate.

### Positive Test

Evolution of a carbon dioxide gas is a positive test for the presence of the carboxylic acid and certain phenols listed in the Complications section.

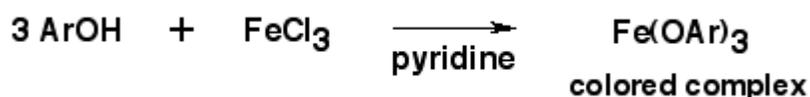
### Complications

Negatively substituted phenols such as nitrophenols, aldehydophenols, and polyhalophenols are sufficiently acidic to dissolve in 5% sodium bicarbonate.

### Tests for Phenols and Nitro Groups

#### Iron (III) Chloride Test for Water-Soluble Phenols

##### Phenol



##### Procedure (for water-soluble phenols)

The iron (III) chloride test for phenols is not completely reliable for acidic phenols, but can be administered by dissolving 15 mg of the unknown compound in 0.5 mL of water or water-alcohol mixture and add 1 to 2 drops of 1% aqueous iron (III) chloride solution.

##### Positive Test

A red, blue, green, or purple color is a positive test.

#### Iron(III) Chloride - Pyridine Test for Water-Insoluble Phenols

##### Phenol



##### Procedure (for water-insoluble phenols or less reactive phenols)

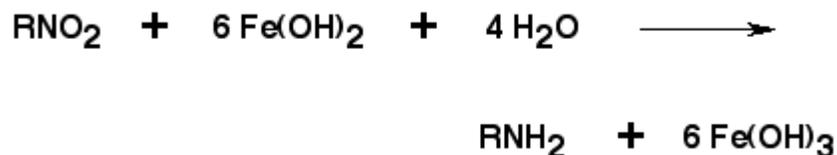
A more sensitive test for phenols consists of dissolving or suspending 15 mg of the unknown in 0.5 mL of methylene chloride and adding 3-5 drops of a 1% solution ferric chloride in methylene chloride. Add a drop of pyridine and stir.

##### Positive Test (b)

Addition of pyridine and stirring will produce a color if phenols or enols are present.

## Iron (II) Hydroxide Test for Nitro Groups

### Nitro Compounds



### Procedure

Add about 10 mg of the compound to 1 mL of the ferrous ammonium sulfate reagent in a test tube, and then add 0.7 mL of the 2N alcoholic potassium hydroxide reagent. Stopper the tube, and shake. Note the color of the precipitate after 1 minute.

*Ferrous Ammonium Sulfate Reagent:* To 50 mL of recently boiled, distilled water add 2.5 g of ferrous ammonium sulfate crystals and 0.2 mL of concentrated sulfuric acid.

The Alcoholic Potassium Hydroxide Reagent will already be prepared for you.

### Positive Test

A positive test is the formation of the red-brown precipitate of iron(III) hydroxide.

### Amines

#### Hinsburg Test for Amines.

If you have a basic compound which you believe to be an amine, you can corroborate your suspicion and determine if you have a primary, secondary, or tertiary amine using the Hinsburg test. You will react the amine with a sulfonyl chloride forming an insoluble sulfonamide of a primary or secondary amine or the soluble salt of a tertiary amine. The insoluble sulfonamide of a primary amine will be made soluble in base (via removal of the slightly acidic proton on N) but that of a secondary amine will not (no proton on N to remove).

### Procedure

Add 100 mg of a solid or 0.1 mL of a liquid unknown, 200 mg of *p*-toluenesulfonyl chloride, and 5 mL of 10% KOH solution to a clean test tube. Stopper the tube and shake it for several minutes. Remove the stopper and heat the mixture on a steam bath for 1 minute. Cool the solution and if it is not basic to pH paper, add additional KOH solution. If a precipitate has formed, add 5 mL of water and shake vigorously. If the precipitate does not redissolve in the basic solution, it is indicative of a sulfonamide of a secondary amine. If there is no precipitate, add 5% HCl until the solution is just acidic when tested by pH paper. Formation of a precipitate under acidic conditions suggests that the previously soluble sulfonamide was of a primary amine. If no precipitate has formed, the initial amine could have been tertiary.

## Synthesis of Acetanilide from Aniline

**OBJECT:** To synthesize Acetanilide from Aniline

Chemicals Required:

Aniline	- 5 ml
GL acetic acid	- 15 ml
Zinc dust	- 2 g

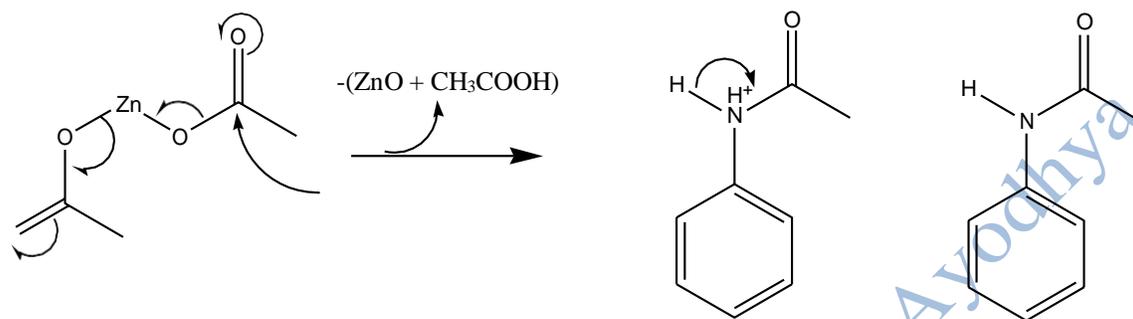
1. A mixture of aniline (5 ml) and zinc dust (2.0 g) in acetic acid (15 ml) in a 100 ml round bottom flask was heated over a gentle flame using water condenser. Heating was continued for about 2 hrs.
2. The reaction mixture was then carefully poured in cold water (100 ml) in a 250 ml beaker with cooling and vigorous stirring. The shining crystals of acetanilide were separated slowly.
3. After 15 min. the acetanilide crystals were collected by filtration. The solid crystals were washed over the Buchner funnel with water and the product was dried (yield, 10 gm). It

was crystallized in boiling water.

M.p. 114 °C.

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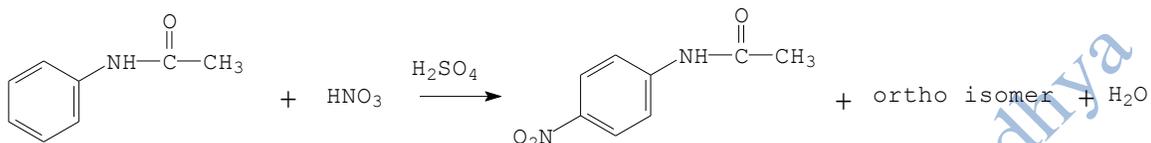
Mechanism:



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## Synthesis of *p*-Nitroacetanilide

OBJECT: To synthesized *p*-Nitroacetanilide Acetanilide from Aniline



### Chemicals Required:

Acetanilide- 5g  
Conc. Sulphuric Acid - 16 ml  
Conc. Nitric Acid – 5ml

### PROCEDURE:

1. Place 1.5 g of acetanilide in a 250-mL RBF flask.
2. Add slowly about 10 mL of concentrated sulfuric acid to the acetanilide. Dissolve most of the solid by swirling and stirring the mixture. Do not be concerned if a small amount of un dissolved solid remains. It will dissolve in later stages of this procedure. Place the flask in an ice bath.
3. Place 5 ml of concentrated nitric acid in another small flask and add about 6ml of concentrated sulfuric acid to it.
4. After addition of acids, swirl the mixture thoroughly in the ice bath. Do not allow the flask to become warm. After 30 minutes, including the time required for adding the nitric acid-sulfuric acid mixture,
5. Pour the reaction mixture in ice cold water. A suspension of *p*- nitroacetanilide is obtained. Allow this mixture to stand for 5 minutes, with constant stirring.
6. Isolate the crude solid by vacuum filtration and recrystallize this solid from water. Allow the crystals to air dry.
7. When the crystals are dry, weigh them and obtain a melting point.

m. p. 214<sup>0</sup>C