**Experiment 1**

**Safety Information-Assessment of Hazards for Experiment**

The chemicals listed below will be used in this experiment. The likely hazards associated with each of the chemicals are noted and recommended procedures for handling are given.

Read this page and the Experimental description carefully before starting the experiment and before coming into the laboratory, noting any potential hazards. When you are satisfied that you understand any possible difficulties that might arise and the recommended procedures for dealing with them, sign the declaration and have it initialled by a demonstrator. Be sure to request information or help if you are in doubt on any point.

|  |  |  |
| --- | --- | --- |
| **Chemical** | **Hazard** | **Precautions** |
| 1,3,5-tribromobenzene | May cause long lasting harmful effects to aquatic  life | Dispose of correctly – do not wash down the sink |
| 4-Bromoaniline | Toxic | Avoid skin contact, wear gloves |
| Benzoic acid | Sensitiser, irritant | Avoid skin contact |
| Silica | Possible carcinogen and sensitiser – but not at the  size particles we are using |  |
| Sodium hydroxide | Corrosive, toxic | Avoid skin contact, wear gloves, complete eye protection **essential**  when handling solutions |
| Hydrochloric acid | Corrosive | Avoid skin contact, avoid  inhaling fumes |
| Dichloromethane | Toxic, irritant | Avoid skin contact, avoid inhaling vapour |
| Sodium sulfate | Low toxicity |  |

NOTE:- When diluting concentrated acids, you should ALWAYS add the acid to water slowly and with mixing. NEVER add the water to the acid. This is because a lot of heat is generated. If you add a small amount of water to concentrated acid it can reach boiling point and splash dangerously.

Declaration - I have read and understood the contents of the Safety Information sheet for this experiment and also the script for the Experiment

S igned (student): ……………………………………………………………………………...

C hecked (demonstrator): …………………………………………………………………….

Date: ...........................................................................................................................

## EXPERIMENT 1: Separation of an Organic Mixture

To be performed on your own

## AIMS of EXPERIMENT

* + - * To separate a complex mixture
      * To investigate the purity of the isolated components using melting point and TLC analysis

## Introduction

When a drug is synthesised, extracted from a natural source or prepared by fermentation, it is unlikely to be pure enough for use. It will need to be separated from a number of experimental by-products or from cell components. Luckily these components are likely to have different chemical properties to the drug, for example the drug might be an acid and the impurity might be a neutral compound, and we can use these differences to separate them.

This experiment focuses on using two of the most simple purification methods to separate four compounds. One compound is inorganic and the other three are an organic neutral compound, an organic acid and an organic base respectively.

You will use filtration methods to remove the inorganic impurity and aqueous / organic separations to separate the organic material. This latter separation method relies on the principles of partition coefficients and on the degree of ionisation of the individual components.

You will use two techniques to assess the success of your separation process in achieving pure compounds. Thin Layer Chromatography and melting point analysis are both powerful and quick techniques for assessing the purity of a compound.

## Experimental procedure



1. **Separation of the inorganic impurity**

The mixture of compounds to be separated is provided. This contains 1,3,5- tribromobenzene, 4-bromoaniline, benzoic acid and silica.

* + Weigh out 3 g of this mixture and record the exact weight in your laboratory notebook.
  + Add this solid to 40 cm3 of dichloromethane (dispensed from the bottle in the fume hood) in a 100 cm3 conical flask and stir. After 5 minutes, the organic components will have dissolved leaving the inorganic impurity as a solid.
  + Filter the mixture through a Büchner filter using suction filtration. Wash the residue with 10 cm3 of dichloromethane, transfer the solid to a labelled watch glass and allow to air dry. YOUR DEMONSTRATOR WILL SHOW YOU THIS TECHNIQUE; IF YOU ARE UNSURE AT ANY TIME CONSULT THEM.

o Once the solid is dry record the mass obtained in your laboratory notebook and ask your demonstrator to initial the notebook.

## Separation of 4-bromoaniline

* + Take the organic solution and pour it carefully into a separating funnel held in a ring on a retort stand.
  + Add 25 cm3 of 2 M hydrochloric acid and stopper the funnel. Holding the stopper securely in place with the palm of your right hand and with the tap in your left hand, invert the funnel and gently shake it. Immediately, with your left hand, release the pressure in the flask by opening the tap. Close the tap and shake the vessel again and release the pressure as before. YOUR DEMONSTRATOR WILL SHOW YOU THIS TECHNIQUE; IF YOU ARE UNSURE AT ANY TIME CONSULT THEM.
  + Place the separating funnel back on the stand and allow the layers to separate. Remove the stopper and run off the organic layer into a 100 cm3 beaker and run off the aqueous layer into a 100 cm3 conical flask and label the flask – **Acidic extract flask A**.
  + Return the organic layer to the separating funnel and add 15 cm3 of 6 M hydrochloric acid. Repeat the separation process and combine the aqueous acid extract with that in Flask A.

## Separation of Benzoic acid

* + Return the organic layer to the separating funnel and extract it twice with 10% sodium hydroxide (2 x 15 cm3).
  + Combine these two aqueous basic extracts in a second 100 cm3 conical flask and label it – **Basic extract flask B**.

## Isolation of 1,3,5-tribromobenzene

* + Transfer the organic layer into a 100 cm3 conical flask and dry it by adding anhydrous sodium sulphate (a demonstrator will show you how to do this). Over 3 minutes, occasionally swirl the flask to allow the sodium sulphate to come into contact with any water present.
  + Filter the solution through a fluted filter paper into a PRE-WEIGHED 250 cm3 round bottom flask. Evaporate the solution to dryness using the rotary

evaporator. YOUR DEMONSTRATOR WILL SHOW YOU THIS TECHNIQUE; IF YOU ARE UNSURE AT ANY TIME CONSULT THEM.

* + - Once the solid is dry record the mass obtained in your laboratory notebook and ask your demonstrator to initial the notebook.

## Isolation of 4-bromoaniline

* + Place flask A into an ice bath formed of ice and water. To free the 4- bromoaniline from its salt, add 6 M sodium hydroxide (NaOH) solution carefully to flask A until the solution is basic (monitor with pH paper YOUR DEMONSTRATOR WILL SHOW YOU THIS TECHNIQUE)
  + Filter the precipitate that forms using suction filtration. Transfer the solid onto a watch glass and allow it to air dry in the fume hood.
    - Once the solid is dry record the mass obtained in your laboratory notebook and ask your demonstrator to initial the notebook.

## Isolation of Benzoic acid

* + Place flask B into an ice bath formed of ice and water. Add 6 M hydrochloric acid (HCl) solution carefully to flask B until the solution is acidic (monitor with pH paper).
  + Filter the precipitate that forms using suction filtration. Transfer the solid onto a watch glass and allow it to air dry in the fume hood.
    - Once the solid is dry record the mass obtained in your laboratory notebook and ask your demonstrator to initial the notebook.

## Thin Layer Chromatography

You were introduced to the technique of Thin Layer Chromatography (TLC) in Semester 1. In this experiment you will use the technique to analyse the mixture and assess the purity of the organic compounds you have isolated.

* + Dissolve about 10 mg (the tip of a spatula) of the mixture of organic components in 1 cm3 of methanol in a test-tube.
  + Take a TLC plate, draw a feint **pencil** line 1 cm from one end and mark 4 crosses on this line.
  + Using a capillary tube apply one spot of the mixture to the first cross and allow the solvent to evaporate.
  + Repeat this process for the 3 components you have separated (acidic, basic and neutral compounds). DO NOT ANALYSE THE INORGANIC IMPURITY. Ask your demonstrator to check you have loaded enough compound onto the plate.
  + In the fume hood, add dichloromethane to the chromatography jar up to a level of approximately 5 mm. With tweezers, place the plate into the jar so that the line on the plate is above the level of the solvent and place the lid on the jar. When the solvent has almost reached the top of the plate remove the plate from the jar and mark the position of the solvent front with by drawing a line WITH A PENCIL across the plate. Allow the solvent to evaporate and then observe the plate under UV light, mark any spots that can be observed WITH A PENCIL. Then place your plate in one of the iodine jars provided, after 5 minutes, mark any spots than can be observed.
    - Prepare a table detailing which spots are observed under which conditions and their R*f* values (R*f =* distance travelled by spot / distance travelled by solvent front)
    - Record the results within the table and ask your demonstrator to initial your notebook

## Melting Point Measurement

The melting points of benzoic acid, 4-bromoaniline and 1,3,5-tribromobenzene should be determined using the apparatus provided. YOUR DEMONSTRATOR WILL SHOW YOU THIS TECHNIQUE; IF YOU ARE UNSURE AT ANY TIME CONSULT THEM.

Remember the melting point of a compound is a range, from when the compound first begins to melt until all is melted. The less pure a compound is the larger the melting point range will be.

* Record the values for the melting range in your laboratory notebook (which should be referenced) for each of the organic compounds and comment on the results

## WRITE-UP

At the end of the experiment, make sure you have included in your lab notes:

* The mass of each compound isolated, a relative ratio of the components of the mixture, and the total mass isolated with a brief comment on why this is or is not the same as the starting mass.
* An explanation, with equations and “CURLY ARROW” mechanisms, for the separation of the organic substances.

## USEFUL REFERENCE

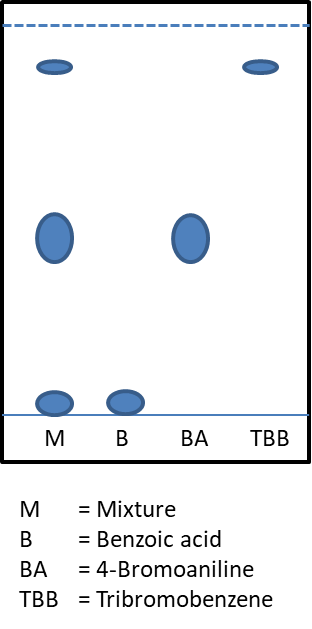
Fundamentals of Organic Chemistry, John McMurry, Thomson, ISBN 05343958the

The result for the lap report

**Masses Obtained**

|  |  |
| --- | --- |
|  | **Masses of each component recovered** |
| Inorganic impurity | 0.4 |
| 4-bromoaniline | 0.1 |
| Benzoic acid | 0.9 |
| 1,3,5-tribromobenzene | 1.3 |
| Total mass of components isolated | 2.7 |

**TLC Results**



**Melting Point Results**

|  |  |
| --- | --- |
| **Compound** | **Observed Melting Point Range** |
| Benzoic Acid | 118-122 °C |
| 4-Bromoaniline | 53-56 °C |
| 1,3,5-Tribromobenzene | 115-118 °C |
|  |  |

**Abstract:**

**[5 marks]**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **RMM**  (relative  molecular mass)  of reactants | **Weight obtained** | **Molar ratio  of reactants** |
| Inorganic impurity |  |  |  |
| 4-bromoaniline |  |  |  |
| Benzoic acid |  |  |  |
| 1,3,5-tribromobenzene |  |  |  |
| Total mass of components isolated |  | | |
| **Comments** |  | | |

**[10 marks]**

**Procedure Notes:**

**[15 marks]**

**Melting point analysis:**

Record the melting point of your purified products below and comment on your results.

**[5 marks]**

**Thin Layer Chromatography Diagram**

Draw your TLC plate in this section and annotate your drawing to explain your results

**[15 marks]**

**R*f* Table**

Prepare a table detailing which spots are observed under which conditions and their R*f* values (R*f =* distance travelled by spot / distance travelled by solvent front)

**[5 marks]**

**Mechanism:**

Include a mechanism for each step in the separation process. Annotate your mechanism to explain what is happening in each step.