University of Paisley

Department of Chemistry and Chemical Engineering

Organic Chemistry Laboratory Report

Separation and Identification of Organic Unknowns

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Industrial Chemistry

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Aim

The purpose of this laboratory experiment was two-fold, first, to demonstrate the extraction of acidic, basic and neutral components from a crude product, finally determining what unknowns were present using some common analytical procedures and techniques.

Introduction

An organic crude product obtained from a 'worked up' reaction mixture will in almost all cases need to be purified further. The work-up, by which this procedure is usually known, simply refers to the isolation of the product from the reaction mixture, free from solvent and spent reagents, and does not imply any purification.

In order to purify an organic compound by separating the impurities, one has to rely on the desired compound having different properties to the impurities. Differences that may be taken advantage of are: differences in solubility, volatility, polarity, shape and functional groups present. For example, crystallisation relies on the differences in solubility between the desired compound and the impurities whereas distillation exploits differences in volatility. Adsorption chromatography separates and purifies compounds according to their adsorption to the chromatographic material, which to a good approximation is related to the polarity of the compounds. Major purification techniques relevant to the laboratory include extraction, crystallisation, distillation and chromatography in all their various forms.

Extraction in the chemical sense means 'pulling out' a compound from one phase to another, usually from a liquid or a solid to another liquid. In the organic laboratory, the most common process involves the extraction of an organic compound from one liquid phase to another. The two liquid phases are usually an aqueous solution and an organic solvent, and the technique is known as *liquid-liquid extraction* or more commonly, as extraction.

A simple extraction is often used in the work-up of an organic reaction mixture, but extraction can also be used to separate and purify organic compounds. Extraction is particularly useful in the separation of water-soluble products (inorganic salts) from water insoluble products (organic compounds). This is usually achieved by taking up the crude product in an organic solvent and extracting the organic solution with water – a procedure usually described as 'washing' the organic solution with water.

Washing a solution is the same as 'extracting' a solution. The difference is that the 'washings' remove undesired material, whereas the 'extractions' remove desired material.

Extraction of the acidic and basic components of a given organic mixture can then be achieved by their reaction with a dilute aqueous base or acid as appropriate. Since this relies on an acid-base chemical reaction, the technique is often called *chemically active extraction*. An extraction protocol for the separation of acidic (AH), basic (B:) and neutral (N) components of a mixture is shown on the following page.

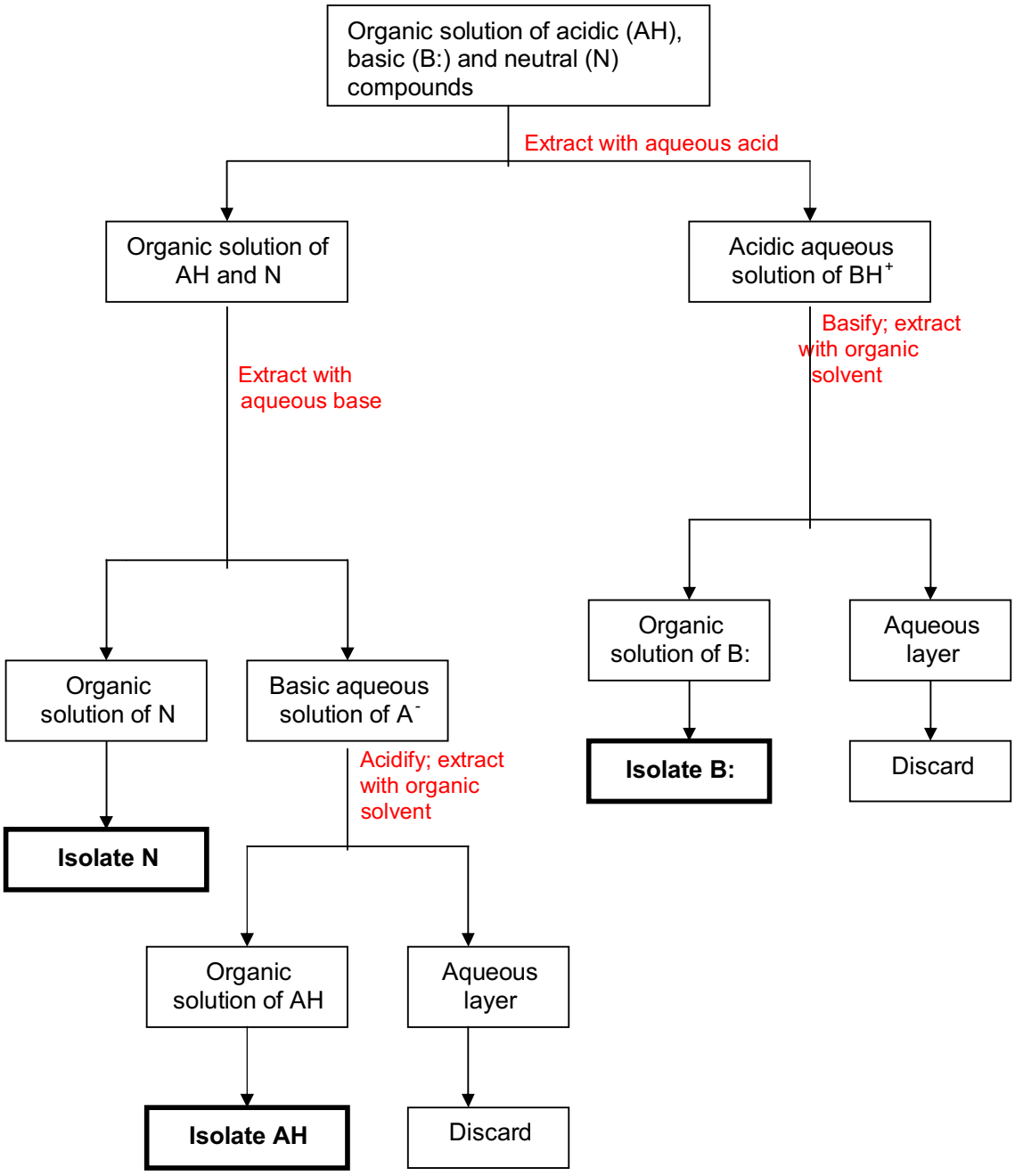


Figure 1. Extraction protocol for the separation of acidic (AH), basic (B:) and neutral (N) components of a crude organic mixture.

Experimental Method

Phase 1: Separation of the organic mixture

The first procedure carried out was to identify a suitable solvent in order to dissolve the crude organic product. The solvent used to dissolve the crude product was dichloromethane as it fulfilled all the main requirements for an extraction solvent: immiscibility with water, different density of water, solubility characteristics, a good stability and volatility so that it can easily be removed from the organic compound by evaporation. Ideally an extraction solvent should also be non-toxic and non-flammable, but these two criteria are less easy to meet. The volume of solvent used to dissolve the organic sample was kept to a minimum (~110ml was used for 11.08g of crude product). The resultant organic solution was then placed in a separating funnel of suitable size where it was washed with water to remove any inorganic salts present. This procedure was repeated twice to ensure all the inorganic salts were removed.

The next separation was to extract organic amines in the mixture by adding dilute hydrochloric acid to the separating funnel. Any organic amines present would now be in the aqueous top layer because they are now present as water-soluble salts. This process was repeated twice in order to make sure all the organic amines present were extracted from the mixture. The resulting aqueous solutions containing the extracted amines were combined and donated as solution (A). The organic solution/phase left was then washed twice with water to remove any traces of hydrochloric acid.

The organic solution was then extracted with dilute sodium hydroxide in order to remove any carboxylic acids. As with extracted amines, any carboxylic acids present in the solution should be present as water-soluble carboxylic acid salts in the aqueous top layer. This procedure was repeated twice in order to remove all carboxylic acids from the solution. The resulting aqueous solutions containing the carboxylic acids were combined and donated as solution (B). The organic solution/phase was then dried by putting it into a conical flask and adding sodium sulphate (anhydrous salt) until the solid became ‘powdery’. Any solvent present was then removed using a rotary evaporator. After the solvent was removed, sticky oil remained and was labelled as product (C). After a week the oil solidified into a brown solid.

Solutions (A) and (B) were then worked up in order to isolate the amines and carboxylic acids present in their respective solutions. Firstly, the amines were isolated from solution (A) by adding 50% sodium hydroxide to the hydr ochloric acid solution containing organic amines as a salt. The sodium hydroxide was added drop wise with stirring until the solution was basic to pH paper (it should be noted that if the molecular weight of the amine base was unknown then it could have been calculated from acid-base titration). The amines present were no longer soluble in the solution and precipitated out as a white solid.

The solid was then filtered off and washed with water to remove any traces of sodium hydroxide. The solid was then allowed to air dry for a week and labelled as product (A).

The carboxylic acids were removed by a similar procedure except that hydrochloric acid was used instead of sodium hydroxide (again it can be noted that if the molecular weight of the carboxylic acid was unknown then it could have been calculate from the acid-base titration). The carboxylic acid also precipitated out as a white solid, which was then filtered and washed with water to remove any traces of hydrochloric acid still present. The carboxylic acid was then allowed to air dry for a week and labelled as product (B).

Phase 2: Thin layer chromatography

The crude organic sample provided at the start of the experiment had now been separated into its constituent components. However it was unclear whether these components were present as a single product or mixture requiring purification. Thin layer chromatography (TLC) provided an analytical technique for determining the purity of the constituents and also for preliminary identification purposes.

A very small quantity of each sample was 'spotted' onto the baseline of a TLC plate (solid adsorbent) and the solvent was allowed to evaporate rapidly through the absorbent layer by capillary action, leaving the small spot of solute behind. The three individual spots (acid, base and neutral sample) moved up with the solvent at different relative rates. These rates depend on a number of factors, including chemical natures of components of the sample being analysed, the nature of the solvent and the activity of the solvent. Ideally the spots should be less than half way up the plate after running the sample.

After testing various solvent systems, methanol-dichloromethane was found to be the most efficient system and from this it was concluded that all three individual components required purification. Solvent polarity is extremely important, with spots moving faster in more polar solvents. Thus polar solvents should be used for strongly absorbed (polar) compounds, and non-polar compounds should be used for weakly absorbed (non-polar compounds).

The chromatogram generated was then exposed to UV light to determine the distance travelled by the spots. The distance travelled by the centre of a particular spot from the starting point where it was applied, divided by the distance travelled by the solvent front upward from the point of spot application, is called the R_f value and is fairly constant for a given compound chromatographed in a given way. Thus, R_f values may be used as aids in identification; responses to visualizing techniques also assist in such identifications.

The visualized spots are almost always larger than they were before development, owing to diffusion. If the spots were too large (as observed during this analysis), the centres are difficult to locate precisely; the spots may even overlap or merge. In this case the TLC run was repeated using the same solvent system and under the same conditions but a smaller sample/spot was applied.

Phase 3: Purification of the products

As the three products obtained were all solids the purification process carried out was crystallisation (known also as recrystallisation). The outline followed for the purification of each organic compound by crystallisation is shown below.

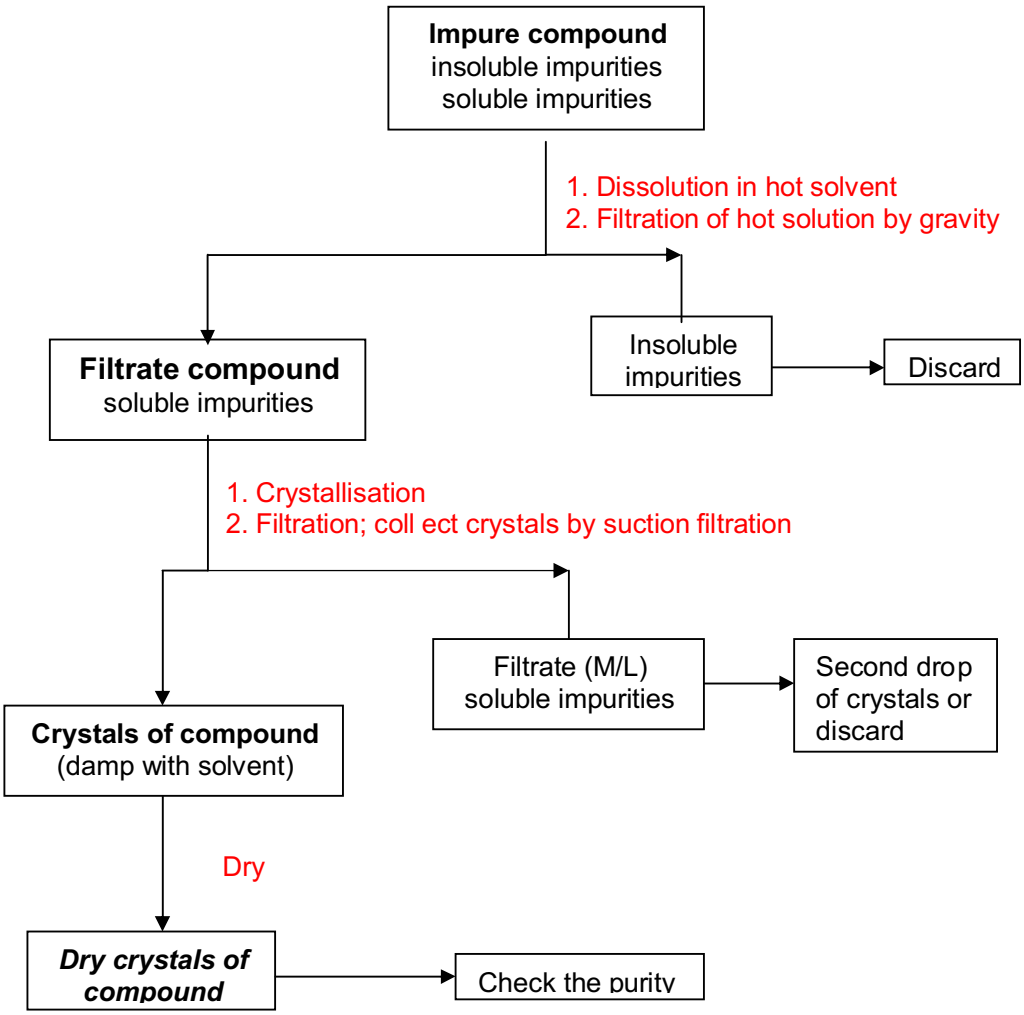


Figure 2. Plan for purification of an organic compound by crystallisation.

The first step in crystallisation is to find a suitable solvent to dissolve the product. Generally choosing a solvent for crystallisation tends to follow the rule 'like dissolves like'. Therefore a nonpolar solvent would be used to crystallise a nonpolar substance and compounds containing polar groups such as OH are best crystallised from polar OH containing solvents.

The ideal solvent for a crystallisation should not react with the compound, should be fairly volatile so it is easy to remove from the crystals, should have a boiling point that is lower than the melting point of the compound to be crystallised, should be non-toxic and non-flammable, but most important of all, the compound should be very soluble in hot solvent and insoluble in cold solvent. A range of solvents were tested by adding a small sample amount to a test tube containing solvent. Pet.Ether was found to best fit the characteristics of a suitable solvent i.e. the sample did not dissolve at room temperature but did dissolve on heating and the crystals reformed on cooling.

A weighed amount of product A was then dissolved in the minimum volume of hot solvent (weighing the solid allowed the % recovery of the material from the crystallisation process to be determined). The solution, together with any soluble impurities was then filtered to remove any insoluble impurities or pieces of extraneous material such as dust or glass. The resulting saturated solution was then allowed to cool at room temperature (side of the beaker was scratched with a glass rod to produce micro-fragments of glass which serve as nuclei to induce crystallisation). The rate of cooling determines the crystal size, rapid cooling favouring the growth of a lot of small crystals, and slow cooling encouraging the growth of fewer, but larger crystals.

It should be noted that if the beaker containing the saturated solution was placed in an ice bath and cooled too quickly then impurity formation would have occurred. This is because the process of crystallisation is equilibrium: molecules in solution are in equilibrium with those in a crystal lattice.

Since a crystal lattice is highly ordered, other different molecules, such as impurities, will be excluded from the lattice and will return to the solution. Therefore only molecules of the required compound are retained in the crystal lattice and the impurities will remain in solution. For a crystallisation to be successful, the solution must be allowed to cool slowly, so that the crystals are formed slowly, and the equilibrium process, which excludes the impurities, is allowed to operate. If a solution is cooled rapidly, impurity molecules will be trapped or included in the rapidly growing crystal lattice. This rapid formation of solid material from solution is precipitation and is not the same as crystallisation.

The crystals were then separated from the *mother liquor* by suction (vacuum) filtration and washed with a small volume of fresh solvent (Pet.Ether). It should also be noted that the mother liquor from the crystallisation (now the filtrate) might contain a significant amount of organic product and therefore reduce the percentage recovery (yield) of the product.

[In this case a second batch of crystals, known as the 'second crop', can often be obtained by concentrating the mother liquor by boiling off some of the solvent and then allowing the solution to cool and crystallise as before. However, the second crop is usually less pure than the first simply because the impurities were concentrated in the M/L during the first crystallisation]

The crystals were then dried in an oven and weighed using an analytical balance. This procedure was repeated for products B and C. A purity test of each component was then carried out by running a TLC. Finally the components of the mixture were identified from the infrared spectra obtained, elemental analysis and ^{1}H-NMR of each product given.

Results and Discussion

	C	H	Cl	N	O	M.Wt.
Acid	68.8	4.90	-	-	*26.3	122
Base	56.42	4.68	27.78	11.01	-	127.5
Neutral	85.80	5.45	-	-	*8.75	182

Table 1. Elemental Analysis and molecular weights of unknowns.

* Must be oxygen to add up to 100 %

The three individual components of the crude mixture can be identified from using analytical techniques. All three compounds in this section of the report were identified by using empirical formula calculations, IR spectra, ^{1}H NMR and melting point determinations. For the unknown compound labelled as 'B' the isoteric formula and ^{13}C NMR were briefly described as additional identification techniques.

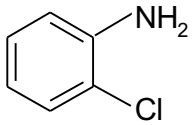
Product A – Unknown Base Compound

Empirical formula: -

Atom	C	H	Cl	N
% composition	56.42	4.90	27.78	11.01
⁒ by atomic no.	12.011	1.008	35.5	14
⇒	4.697	4.861	0.783	0.786
⁒ by smallest no.	(0.783)	(0.783)	(0.783)	(0.783)
⇒	6.00	6.21	1.00	1.00
approximate	**6**	**6**	**1**	**1**

Empirical formula ⇒ $\mathbf{C_6H_6ClN}$, which corresponds to molecular weight of 127.5 amu.

∴ Unknown basic compound could be *Chloroaniline*.



IR analysis: -

Functional group	Absorption	Theoretical range	Observed peaks
Aromatic	υ Ar-H	3040-3010 cm^{-1}	3030 cm^{-1}
	υ C=C	1600,1580,1500,1450 cm^{-1}	1620,1490 cm^{-1}
	υ Ar-H (para-disub)	860-800 cm^{-1}	820 cm^{-1}
Amine (1°)	υ N-H	3500-3300 cm^{-1}	3500-3350 cm^{-1}
	δ N-H	1650-1560 cm^{-1}	1620 cm^{-1}
	υ Ar-N	1360-1250 cm^{-1}	1280 cm^{-1}

Infrared spectra confirms that the basic compound is Chloroaniline.

^{1}H NMR analysis: -

The expected chemical shifts for the 1 H NMR spectrum for chloroaniline were calculated and compared to the 1H NMR spectrum for the base component given at the start of this experiment.

(3 signals expected)

	Expected			Observed		
Protons	**Chemical Shift**	**Coupling pattern**	**Integration**	**Chemical shift**	**Coupling pattern**	**Integration**
a	7.05	multiplet	2	7.10	multiplet	2
b	6.50	multiplet	2	6.60	multiplet	2
c	3.0-6.0	singlet	2	3.60	singlet	2

Calculations: -

$\delta\ \delta H^a = 7.27 + \text{ortho (Cl)} + \text{meta } (NH_2) = 7.27 + 0.03 + (-0.25) = 7.05$ ppm

$\delta\ \delta H^b = 7.27 + \text{ortho (NH2)} + \text{meta (Cl)} = 7.27 + (-0.75) + (-0.03) = 6.50$ ppm

The ^{1}H NMR confirms the structure of **chloroaniline**.

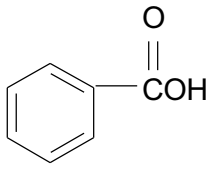
Product B – Unknown Acid Compound

Empirical formula: -

Atom	**C**	**H**	**O**
% composition	68.8	4.90	26.3
⁒ by atomic no.	12.011	1.008	15.999
$\Rightarrow$	5.728	4.861	1.644
⁒ by smallest no.	(1.644)	(1.644)	(1.644)
$\Rightarrow$	3.50	2.96	1.00
X 2 (approximate)	**7**	**6**	**2**

Empirical formula $\Rightarrow$ $\mathbf{C_7H_6O_2}$, which corresponds to molecular weight of 122 amu.

$\therefore$ Unknown acid compound could be *benzoic acid*.



Number of double bonds: -

Molecular formula is $C_7H_6O_2$ – replacing with CH_2 for oxygen to give isoteric formula i.e. $C_2H_4 + C_7H_6 = C_9H_{10}$

Saturated open chain would give formula = C_9H_{20}

Difference of 10 hydrogen's = 5 double bond equivalents. This is high for a molecule of this molecular weight and suggests the presence of an aromatic ring (4 double bond electrons) plus an unsaturated group. The absence of nitrogen rules out functional groups such as an amino or nitro group in the acid.

This would offer further evidence that constituent B is a carboxylic acid derivative – benzoic acid.

IR analysis: -

Functional group	Absorption	Theoretical range	Observed peaks
Aromatic	υ C=C	1600, 1580, 1500, 1450 cm^{-1}	1603 and 1582 cm^{-1}
	δ Ar-H (mono substituted)	770-730 & 720-680 cm^{-1}	700 cm^{-1}
Carboxylic acid	υ O-H	3200-2500 cm^{-1}	3100-2500 cm^{-1}
	υ C=O (aromatic)	1700-1680 cm^{-1}	1695 cm^{-1}
	δ O-H or υ C-O	1320-1220cm^{-1}	1287 cm^{-1}

^{1}H NMR analysis: -

The expected chemical shifts for the ^{1}H NMR for benzoic acid were calculated and compared to the ^{1}H NMR spectrum for the acid component given at the start of this experiment.

(4 signals expected)

Expected				Observed			
Protons	**Chemical Shift**	**Coupling pattern**	**Integration**	**Chemical shift**	**Coupling pattern**	**Integration**	**Coupling constant**
a	7.54	multiplet	3	7.75-7.30	multiplet	3	-
b	7.45	mulitplet	3	7.75-7.30	multiplet	3	-
c	8.12	doublet	2	8.15	doublet of doublets	2	J_{cb} 8.0Hz, J_{ca} 1.0Hz
f	9.0-15.0	singlet	1	12.6	Broad singlet	1	-

Calculations: -
δH^a = 7.27 + para (CO_2H) = 7.27 + 0.27 ppm = 7.54 ppm.
δH^b = 7.27 + meta (CO_2H) = 7.27 + 0.18 ppm = 7.45 ppm.
δH^c = 7.27 + ortho (CO_2H) = 7.27 + 0.85 ppm= 8.12 ppm.

On looking at the IR spectra, there is a strong and very broad O-H absorption, which is very characteristic of a carboxylic acid rather than an alcohol or phenol. The ^{1}H NMR for D_2O exchangeable signals provides a check and here such a signal at 12.6 ppm can be observed which again is typical of a carboxylic acid.
There is a strong absorption at 1695 cm^{-1}, which could be the carbonyl absorption for the said carboxylic acid.
The only other possible carbonyl group is an aldehyde, but on checking the ^{1}H NMR it is clear there is no aldehyde proton present at 9.6-9.9 ppm. Thus, the carbonyl absorption must be due to a carboxylic acid group.
Further absorptions are present at 1603 and 1582 cm^{-1}, which are typical of an aromatic ring.
The presence of an aromatic ring and a carboxylic acid would account for the five DBE's and two oxygen atoms.
At this stage, there is only one structure, which could fit this evidence since the aromatic ring, and carboxylic acid would account for all the atoms in the molecular formula. That structure is **benzoic acid**.

^{13}C NMR analysis: -

^{13}C NMR analysis was not actually carried out in this laboratory experiment, however, the tables below illustrate the expected values which can be calculated against the likely observed values which would be seen for a ^{13}C NMR spectra of benzoic acid.

(5 signals expected)

Carbon	Type	Expected	DEPT	Observed
a	CH	133	CH	133.8
b	CH	128	CH	128.5
c	CH	130	CH	130
d	C	130	C	129.4
e	C	164-169	C	172.7

Chemical Shift Calculations

Carbon	Base value	Effect of $-CO_2H$	Total
a	128	para 5	133
b	128	meta 0	128
c	128	ortho 2	130
d	128	ipso 2	130

There are five carbon signals in the spectrum but seven carbons in the molecular formula of benzoic acid. This implies some level of symmetry in the molecule. All five of the carbons are unsaturated (i.e. greater than 77 ppm). There are two very weak unsaturated carbons, which are likely to be quaternary. The carbon signal at 127.7 ppm must be a carbonyl carbon. The remaining four signals will be the aromatic carbons. The number of signals and the relative intensities are characteristic of a mono-substituted ring. All the information here fits the structure of benzoic acid.

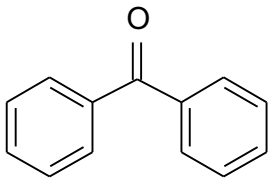
Product C – Unknown Neutral Compound

Empirical formula: -

Atom	C	H	O
% composition	85.80	5.45	8.75
⁒ by atomic no.	12.011	1.008	15.999
⇒	7.143	5.407	0.547
⁒ by smallest no.	(0.547)	(0.547)	(0.547)
⇒	13.06	9.88	1.00
approximate	**13**	**10**	**1**

Empirical formula ⇒ $\mathbf{C_{13}H_{10}O}$, which corresponds to molecular weight of 182 amu.

∴ Unknown neutral compound could be *benzophenone*.



IR analysis: -

Functional group	Absorption	Theoretical range	Observed peaks
Aromatic	υ Ar-H	3040-3010 cm^{-1}	3050 cm^{-1}
	υ C=C	1600,1580,1500,1405 cm^{-1}	1650, 1580 cm^{-1}
	δ Ar-H (ortho disub)	770-735 cm^{-1}	750 cm^{-1}
Ketone	υ C=O (aromatic)	1700-1680 cm^{-1}	1660 cm^{-1}

^{1}H NMR analysis: -

The expected chemical shifts for the ^{1}H NMR for benzophenone were calculated and compared to the ^{1}H NMR spectrum for the neutral component given at the start of this experiment.

(3 signals expected)

	Expected			Observed		
Protons	**Chemical Shift**	**Coupling pattern**	**Integration**	**Chemical shift**	**Coupling pattern**	**Integration**
a	7.48	multiplet	6	7.30-8.00	multiplet	6
b	7.41	multiplet	6	7.30-8.00	multiplet	6
c	7.89	multiplet	6	7.30-8.00	multiplet	6

Calculations: -

δ δH^a = 7.27 + para (COR) = 7.27 + 0.21 = 7.48 ppm

δ δH^b = 7.27 +meta (COR) = 7.27 + 0.14 = 7.41 ppm
δ δH^c = 7.27 +meta (COR) = 7.27 + 0.62 = 7.89 ppm

The ^1H NMR for the neutral compound does not fully confirm the structure of benzophenone. However the infrared provides the evidence needed to show that the neutral compound is infact **benzophenone**.

% Recoveries

Compound	Wt. Before Purification	Wt. After Purification	% Recovery
Chloroaniline	2.04g	1.68g	82.35
Benzoic Acid	3.76g	3.24g	86.17
Benzephenone	1.96g	0.33g	16.84

Melting Points

Compound	Melting Point / ^oC	Literature Value / ^oC
Chloroaniline	71-75	72.5
Benzoic Acid	122	122.4
Benzephenone	48-50	48.5

There are three possible isomers of chloroaniline, m-chloroaniline and p-chloroaniline. The melting point value proves that product A is *p-chloroaniline* because o-chlroaniline and m-chloroaniline both have melting point values under 0^oC and are present at room temperature as liquids. Therefore product A is definitely p-chloroaniline.

Conclusion

It can be deduced that from all the evidence obtained from the analytical techniques used in this experiment, the organic sample provided is made up of *Benzophenone*, *Benzoic Acid* and *p-Chloroaniline*.

This laboratory experiment has also allowed me to gain valuable practical experience of organic work up procedures involving a variety of techniques including extraction, crystallisation, distillation and chromatography. These techniques are particularly useful in the separation and purification of product mixtures or unknown neutral compounds.

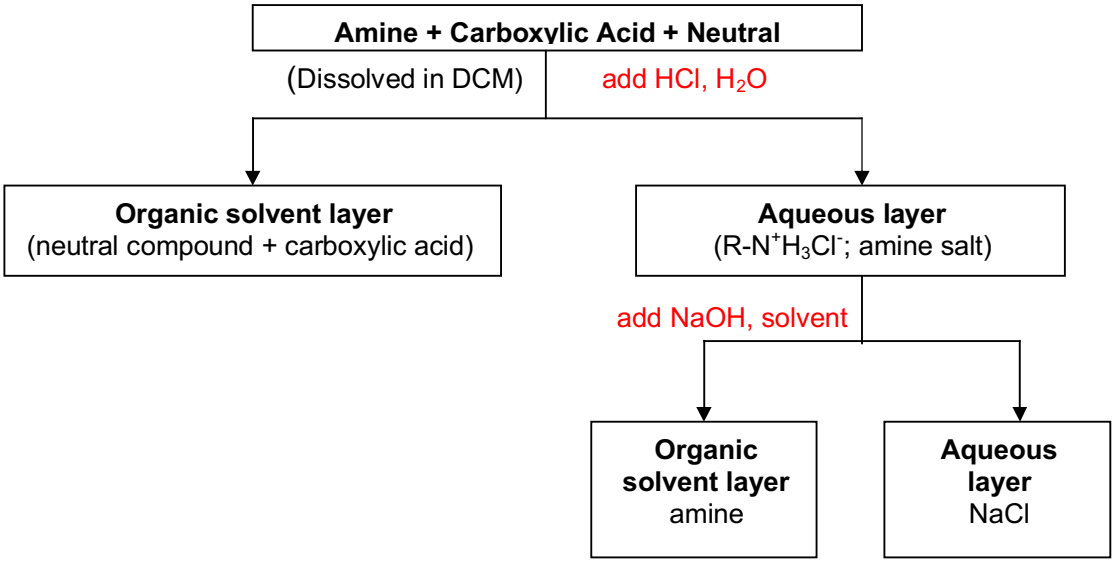
Appendix 1 – Questions

Separation by Extraction

Simple mixtures of organic compounds that are normally soluble in organic solvents such as dichloromethane or ether but insoluble in water can be separated by extraction using reagents such as hydrochloric acid or sodium hydroxide. These reagents selectively react with organic (carboxylic) acid and organic base (amine) components in the mixture to form ions that are soluble in water.

- Base (Amine) – Stages 3,7 and 8

If a mixture of a basic amine, carboxylic acid and a neutral compound such as a ketone or aldehyde are dissolved in an organic solvent and extracted with aqueous acid, the basic amine dissolves in the water layer as its protonated salt, while the neutral and acidic compounds remain in the organic solvent layer. Separation, basification and extraction of the aqueous layer with organic solvent then provide the pure amine as depicted below.



Reaction Scheme:

$R\text{-}NH_2$ + HCl $\longrightarrow$ $R\text{-}NH_3^+$ + Cl^-

amine water insoluble — amine salt, water soluble

$R\text{-}NH_3^+$ + NaOH $\longrightarrow$ $R\text{-}NH_2$ + Na^+ + H_2O

salt of base, water soluble — amine water insoluble

- Acid (Carboxylic acid) – Stage 5, 10 and 8

Carboxylic acids react with bases such as sodium hydroxide to give carboxylate salts. Carboxylic acids with more than six carbons are only slightly soluble in water, but metal salts of carboxylic acid are generally quite water-soluble because of their ionic nature. Its possible to purify acids by extracting their salts into aqueous base, then reacidifying and extracting the pure acid back into an organic solvent.

Reaction Scheme:

$R–CO_2H$ + NaOH $\longrightarrow$ $R\text{-}CO_2^-$ + Na^+ + H_2O

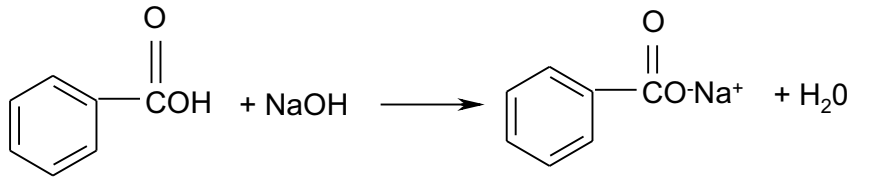
Carboxylic acid (dilute ~ 5M) carboxylate ion water soluble

then

RCO_2^- + HCl $\longrightarrow$ $R\text{-}CO_2H$ + Cl^-

carboxylate ion water soluble carboxylate acid water insoluble

Benzoic acid dissolves in aqueous sodium hydroxide as shown below.



Benzoic acid (water insoluble) stronger acid — stronger base — Sodium benzoate (water soluble) weaker base — weaker acid

- Neutral compound (Stage 6) is an organic compound that is neither an acid nor base remaining in the organic solution.

Appendix 2 – IR spectra

The IR spectra obtained for the three constituents of the crude organic compound are attached to this report in the following order: -

Compound A – Base

Compound B – Acid

Compound C - Neutral