EXTENDED ESSAY
- Chemistry -

How does reflux time affect the yield and purity of ethyl aminobenzoate (Benzocaine), and how effective is recrystallisation as a purification technique for this compound?

Word Count: 3977

AMEERA PATEL

NOVEMBER 2006

How does reflux time affect the yield and purity of ethyl aminobenzoate (Benzocaine), and how effective is recrystallisation as a purification technique for this compound?

Ameera Patel

CONTENTS

Ameera Patel

ABSTRACT

In this experiment I have investigated the research question: "How does reflux time affect the yield and purity of ethyl aminobenzoate (Benzocaine), and how effective is recrystallisation as a purification technique for this compound?"

Benzocaine is synthesised by means of an esterification reaction whereby ethanol is refluxed with 4-aminobenzoic acid using an acid catalyst (H_2SO_4). The reflux time was varied to produce different samples. The amine salt produced was converted to an amine by addition of sodium hydroxide solution, and the acid catalyst was neutralised. The Benzocaine samples were isolated using a Büchner funnel and the yields of the dry samples measured. Finally, melting point apparatus and TLC were used as determinants of purity. A few impure samples were subsequently purified using recrystallisation, and the effects of this procedure noted.

The yield-reflux time graph indicated a maximum yield at a reflux time of 82-90 minutes. Samples near this reflux time had the highest purity (noted from both the melting points and TLC). It is suggested that low yields and purity at short reflux times were associated with unreacted reactants present in the samples. The low yield was associated with sodium hydroxide solution neutralising the 4-aminobenzoic acid resulting in the aqueous salt filtering out. The low purity was a result of reactants present in the final product. Above a reflux time of 60 minutes, the reaction mixture appeared to have reached equilibrium but subsequently decomposed for very long reflux times. The low yields and purity noted were associated with this. It was suggested that the recrystallisation technique using equal volumes of 1-chlorobutane, petroleum ether and propanone, caused the Benzocaine samples to decompose and was therefore not a good purification technique in this case. A reason for this has been suggested in terms of an inappropriate solvent being used.

Word Count: 297

How does reflux time affect the yield and purity of ethyl aminobenzoate (Benzocaine), and how effective is recrystallisation as a purification technique for this compound?

Ameera Patel

INTRODUCTION

Benzocaine (ethyl aminobenzoate) is a white crystalline ester[1], commonly used as a topical pain reliever in many over-the-counter analgesic products.[2] Having seen and experienced the use of local anaesthesia in surgical procedures and having investigated the numerous products on the market containing topical anaesthetic, my interest in this area of chemistry was established early on. I was interested in concentrating my investigation on Benzocaine since it is one of the more common anaesthetics used in over-the-counter medicines.[3] My investigation is specifically focused on the synthesis of Benzocaine and how varying the reflux time of the esterification stage affects the yield. However, I soon asked myself whether the purity of the end product would subsequently be affected with differing reflux times; a short reflux time may leave some reactants unreacted. Hence, I chose to test the purity of the samples created by finding their melting points (and the range over which they melted) and comparing these to literature values. However, this would not tell me which impurities were residual in the final product, so I decided to use thin-layer chromatography (TLC) to test this. I then asked myself whether it would be possible to purify the Benzocaine samples further using recrystallisation to produce a compound more similar to that used commercially. This will be the focus of the second part of my investigation, where I will discuss the effectiveness of this purification technique on the ester Benzocaine.

RESEARCH QUESTION

How does reflux time affect the yield and purity of ethyl aminobenzoate (Benzocaine), and how effective is recrystallisation as a purification technique for this compound?

BACKGROUND INFORMATION

3.1 The Synthesis of Benzocaine

Benzocaine is a topical anaesthetic which has been clinically approved and tested for health risks. It is derived from the compound PABA (*para*-aminobenzoic acid or 4-aminobenzoic acid) and its synthesis involves a Fischer esterification reaction involving refluxing an alcohol (ethanol) with a carboxylic acid (4-aminobenzoic acid) using an acid catalyst (sulphuric acid)[4]. The products are an ester (ethyl aminobenzoate) and water. The esterification process is reversible, and hence equilibrium will be reached. The homogenous proton catalyst enables the formation of an intermediate, and is subsequently regenerated at the end. Hence, to remove the acid, it should be neutralised by a solution of sodium hydroxide ($NaOH_{(aq)}$), and the products filtered under vacuum pressure using a Büchner funnel. The synthesis of amines normally results in the production of

[1] Princeton University. http://wordnet.princeton.edu/perl/webwn?s=benzocaine [31 July 2006]
Pennsylvania State University. http://courses.chem.psu.edu/chem36/Web%20Syn06/Exp86Syn06.pdf [31 July 2006]

[2] EMEA. http://www.emea.eu.int/pdfs/vet/mrls/081101en.pdf [31 July 2006]
LaborLawTalk Dictionary. http://dictionary.laborlawtalk.com/benzocaine [31 July 2006]

[3] The use of Benzocaine extends from first aid creams and sore-throat sprays to sunburn remedies and fish anaesthetic.

[4] Answers corporations. www.answers.com [1 August 2006]
InfoChem . http://www.organic-chemistry.org/frames.htm?http://www.organic-chemistry.org/namedreactions/fischer-esterification.shtm [1August 2006]

amine salts. Hence, $NaOH_{(aq)}$ is also used to convert the amine salt of Benzocaine into Benzocaine. Below are the overall reactions occurring in the synthesis and the mechanism for the esterification reaction.

Overall Reaction: Esterification

$$NH_2C_6H_4COOH + C_2H_5OH + 2H^+ \underset{}{\overset{\text{Reflux}}{\rightleftharpoons}} C_2H_5CO_2C_6H_4NH_3^+ + H_3O^+$$

Overall Reaction: Neutralisation and formation of the amine (Benzocaine)

1. $H_2SO_4 + 2NaOH \longrightarrow Na_2SO_4 + 2H_2O$
2. $C_2H_5CO_2C_6H_5NH_3^+ + OH^- \longrightarrow C_2H_5CO_2C_6H_5NH_2 + H_2O$

Mechanism for Acid Reflux Esterification of 4-aminobenzoic acid with Ethanol [5]

STAGE 1:

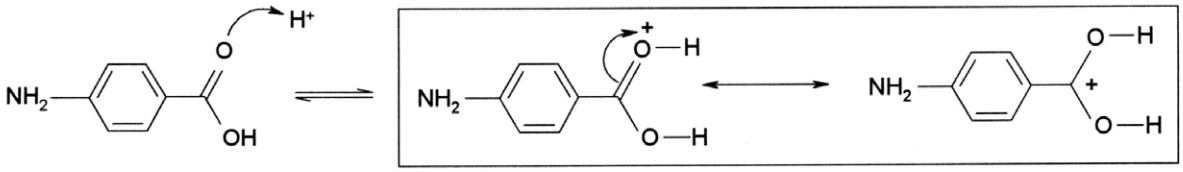


Fig. 1 Protonation – the H^+ ion is dissociated from the acid and attaches to the lone pair on the carbonyl oxygen. A Resonance Hybrid (delocalised structure) is produced where the actual structure lies between the two extremes shown; therefore there is some positive charge on the oxygen and on the carbon atoms [5].

STAGE 2:

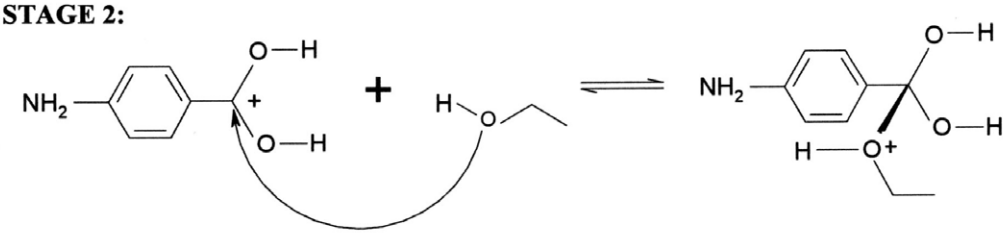


Fig. 2 Alcohol attaches – The positive charge on the carbon attracts the lone pair on the oxygen atom of the alcohol. The structure formed is a tetrahedral intermediate.

[5] Clark, J. http://www.chemguide.co.uk/physical/catalysis/esterify.html [2 August 2006]
MacMillan, J.G http://www.chem.uni.edu/~macmilla/mcmurry/mcmurry_chapter_21/sld017.htm [2 August 2006]
Blackwell Publishing Group http://www.blackwellpublishing.com/11thhour/book6/oc2ch7.html [2 August 2006]

How does reflux time affect the yield and purity of ethyl aminobenzoate (Benzocaine), and how effective is recrystallisation as a purification technique for this compound?

Ameera Patel

STAGE 3:

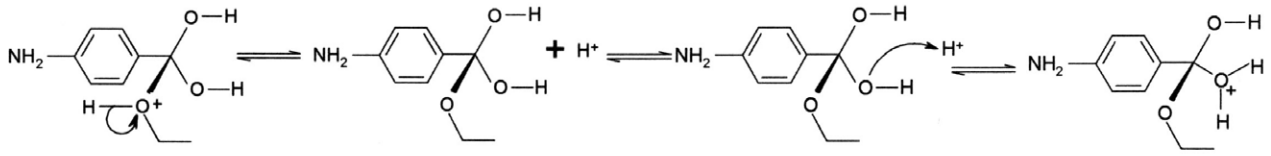


Fig. 3 Rapid Proton Transfer – A hydrogen atom attaches onto another oxygen atom

STAGE 4:

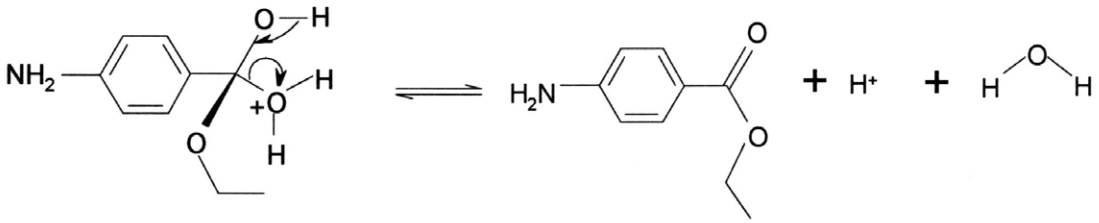


Fig. 4 Loss of water and Acid Regeneration

3.2 Determination of purity using Melting Point Apparatus

Melting Point is a useful measure for the purity of a solid. Since the presence of impurities lowers the melting point and increases the range over which the solid melts, it is easy to see whether the substance is impure. 'Melting Point apparatus' is commonly used for this purpose. It consists of a heated metal block with holes for a thermometer and melting point tubes[6]. The capillary tubes are provided open-ended and require drawing out in a hot flame to close the ends. The crystalline solid can then be transferred into the tube and forced to the bottom by gentle tapping. The compound is heated slowly (c.1 °C per minute [6]) especially around its melting point for accuracy.

[6] Sharp,J.T. Gosney,I. Rowley, A.G. (1989), Practical Organic Chemistry (A student handbook of techniques), London, Chapman & Hall

3.3 Determination of purity using Thin Layer Chromatography

TLC plates consist of a backing material (e.g. plastic) and are coated with an adsorbent (e.g. silica gel). Disposable plates also contain an inorganic fluorescent agent enabling the spots on the chromatogram to show up as blue marks under a low intensity UV lamp[6]. The mixture being tested is dissolved in a solvent and the different compounds (seen as spots under the UV lamp) move different distances up the plate due to their differing affinity for the stationary and mobile phases. Spotters (used to place the solution onto the baseline) can be made by drawing out capillary tubes under a hot flame and breaking them at the restriction.

R_f values (identical for the same compound in the same solvent) can then be recorded, which are calculated by dividing the distance moved by the compound by the distance moved by the solvent front.

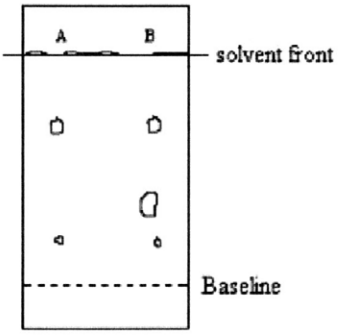


Fig. 5 TLC plate under U-V light.

3.4 Purification of Benzocaine using Recrystallisation

Recrystallisation is a useful purification technique, which operates on the basis that solids are more soluble in hot solvents than in a cold ones[6]. The compound is dissolved until saturated in a boiling solvent (one that the compound dissolves in when hot but not cold) and filtered hot in a Büchner funnel to remove any undissolved impurities. Cooling the filtrate until the compound has completely crystallised out and filtering it again will remove all soluble impurities which do not crystallise out (as they are present in low concentrations). Hence, a purer version of the original compound can be obtained.

[6] Sharp,J.T. Gosney,I. Rowley, A.G. (1989), Practical Organic Chemistry (A student handbook of techniques), London, Chapman & Hall

How does reflux time affect the yield and purity of ethyl aminobenzoate (Benzocaine), and how effective is recrystallisation as a purification technique for this compound?

Ameera Patel

METHOD

[See Appendix I for the full apparatus and chemical lists].

4.1 Synthesising Benzocaine[8]

The sample of Benzocaine had to be produced well in advance of performing tests on its purity, since drying the samples took approximately 8 hours. The first stage of the process was to measure out 20 cm^3 of ethanol into a 250 cm^3 round bottom flask[9]. 3.0 g of PABA was added to the flask and gently swirled (the excess disappeared on refluxing). 3.0 cm^3 of concentrated sulphuric acid was added carefully to the flask and the mixture refluxed to a gentle boil for the specified time. After refluxing, the mixture was cooled to room temperature using a container of cold water. The condenser was then removed and the mixture stirred whilst adding 65-75 cm^3 of $NaOH_{(aq)}$ until the mixture was neutral (tested using pH paper). The mixture was then left to stand for 15 minutes and poured into a 1 dm^3 beaker[10] where it was made up to 500 cm^3 with distilled water and filtered using a Büchner funnel under vacuum. The solid was transferred onto a watch glass and dried in an oven at a temperature below 70°C.

4.2 Testing Melting Points of the samples

Firstly, the sample holders were made. The tube was filled 1 cm high with a sample, placed in the melting point apparatus and heated. The sample was observed under the magnifying lens, and the thermometer was used to record the temperature at which the sample started and finished melting.

4.3 Performing TLC on the samples

TLC was performed on every sample of Benzocaine obtained. For each sample, a solvent was first prepared. Preliminary results showed that it was very difficult to find an appropriate solvent mixture. Since this can only be done by trial and error, it is very time consuming. A solvent that the sample is too soluble in causes the compounds to move too far up the plate (possibly off the plate completely) whilst other solvents that the sample is insoluble in do not move the compounds from the baseline. From preliminary experiments, I found that the samples were very soluble in propanone and 1-chlorobutane (as they are polar), but insoluble in petroleum ether. From trying different combinations of solvents, I found that 1/3 propanone, 1/3 1-chlorobutane, 1/3 petroleum ether worked best. 10 cm^3 of each solvent was mixed to produce a 30 cm^3 mixture[11] and a small amount of this (c.10 cm^3) poured into a 100 cm^3 beaker. A baseline was drawn on a TLC plate 2 cm from the base. Spotters were made and the sample was dissolved in propanone[12] and spotted onto the baseline. The plate was then placed in the beaker and the beaker covered with aluminium foil to minimise evaporation. Once the solvent had reached within 2 cm of the top of the plate, the plate was dried with a hair drier and examined under a UV lamp. R_f values were subsequently calculated.

[8] Personal correspondence: Professor S Ahmed, University of Kingston [20 June 2006]
[9] This flask size was determined from my preliminary experiments where I discovered that c. 60 - 65 cm^3 of $NaOH_{(aq)}$ was required, hence a smaller 100 cm^3 flask (as I had planned to use) would be inappropriate.
[10] Then beaker was in a container full of iced water
[11] Although a smaller quantity of solvent was used for the TLC, the volatile nature of the mixture caused the volume to decrease rapidly even when covered. Therefore, to keep consistency of solvent, a large quantity was mixed.
[12] The samples are readily soluble in propanone

Ameera Patel

4.4 Recrystallisation

As with the TLC, a solvent needed to be found, where the samples were insoluble in cold solvent but soluble in boiling solvent.[13] From trial and error in preliminary experiments, I found that the sample just dissolved in a solution of 93% petroleum ether and 7% propanone at room temperature, hence a solution of 95% petroleum ether and 5% acetone was used. The sample was dissolved in boiling solvent, filtered hot (using a Büchner funnel), and then left to cool so that it would crystallise out. The sample was transferred onto a watch glass and oven dried at a temperature less than 70°C. The tests discussed in sections 7.2 and 7.3 were carried out again to determine whether the sample was pure.

[13] In this case, a mixture of solvents (in different ratios) in which the sample is very soluble and completely insoluble will enable an appropriate solvent to be found.

How does reflux time affect the yield and purity of ethyl aminobenzoate (Benzocaine), and how effective is recrystallisation as a purification technique for this compound?

Ameera Patel

RESULTS

Section 1: Table of Yields of Benzocaine produced for different reflux times

	Reflux Time /min[14]	Mass of filter paper & watch glass[15] /g ± 0.005	Mass of filter paper, watch glass & Benzocaine sample /g ± 0.005	Mass of Benzocaine sample ± 0.01
1	30	45.85	47.63	1.78
2	45 (1)	17.60	19.84	2.24
3	45 (2)	43.37	45.37	2.00
4	60 (1)	43.44	45.74	2.30
5	60 (2)	45.38	47.70	2.32
6	75 (1)	44.30	46.52	2.22
7	75 (2)	22.81	25.09	2.28
8	90 (1)	43.39	45.72	2.33
9	90 (2)	21.36	23.85	2.49
10	105 (1)	43.56	45.70	2.14
11	105 (2)	18.72	21.10	2.38
12	120	44.31	46.47	2.16

Section 2: Table of Melting Points for the samples of Benzocaine

	Reflux Time /min	Minimum Melting Point[16] /°C ± 0.3	Maximum Melting Point[16] /°C ± 0.3	Range of Melting /°C ± 0.6
1	30	80.0	84.5	4.5
2	45 (1)	80.0	84.5	4.0
3	45 (2)	81.0	85.0	4.0
4	60 (1)	81.5	85.0	3.5
5	60 (2)	81.5	85.0	3.5
6	75 (1)	82.5	85.5	3.0
7	75 (2)	82.5	85.5	3.0
8	90 (1)	83.5	86.0	2.5
9	90 (2)	84.0	86.5	2.5
10	105 (1)	82.5	85.5	3.0
11	105 (2)	81.5	85.0	3.5
12	120	81.5	85.0	3.5

[14] The numbers in brackets refer to repeat procedures producing different samples for the same reflux time.
[15] The filter paper from the Büchner funnel was transferred into the watch glass to avoid loss of the sample due to spillage.
[16] The minimum and maximum melting points refer to the temperatures at which the sample starts and finishes melting (respectively).

Section 3: Table of R_f values for the different samples from the TLC[17]

	Reflux Time /min	Distance moved by the solvent front /cm ± 0.2	Distance moved by spot A[18] /cm ± 0.2	Distance moved by spot B[18] /cm ± 0.2	R_f value for spot A ± 0.2	R_f value for spot B ± 0.2
1	30	5.3	2.0	3.9	0.4	0.7
2	45 (1)	5.8	2.2	4.3	0.4	0.7
3	45 (2)	5.7	2.2	4.2	0.4	0.7
4	60 (1)	5.4	2.0	4.0	0.4	0.7
5	60 (2)	5.5	2.1	4.1	0.4	0.8
6	75 (1)	6.0	2.3	4.4	0.4	0.7
7	75 (2)	5.8	2.2	4.3	0.4	0.7
8	90 (1)	5.5	-	4.0	-	0.7
9	90 (2)	5.5	-	4.1	-	0.8
10	105 (1)	5.4	-	4.0	-	0.7
11	105 (2)	5.2	2.0	3.8	0.4	0.7
12	120	5.3	2.0	3.9	0.4	0.7

The R_f value for PABA is 0.38[19]. The assumption here is that spot B is actually Benzocaine.

Section 4: Recrystallisation

The samples created for a reflux time of 105 minutes (sample1) and 60 minutes (sample1) were recrystallised. The melting points and R_f values for these samples can be seen below.

A: 105 minutes (sample1)

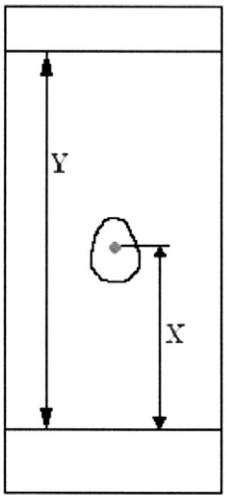


Fig. 6 TLC after recrystallisation of the sample created by a reflux time of 105 minutes

R_f value = $\frac{X}{Y} = \frac{2.9}{5.8} =$ **0.5** ± 0.2

Minimum Melting Point = **77.0°C** ± 0.3
Maximum Melting Point = **82.0°C** ± 0.3

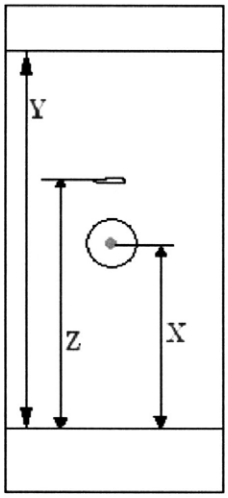
Range of Melting = **5.0°C** ± 0.6

[17] Refer to section 3.3 for details on TLC
[18] Spot A & Spot B refer to the two spots that appear on some TLC plates. Spot A in each case is the one that appears lowest on the plate, and spot B is the one that appears highest on the plate. If only one spot is present, it is always high on the TLC plate, and is therefore classified as spot B.
[19] This value is an average of three values taken (0.377, 0.373, 0.384)

How does reflux time affect the yield and purity of ethyl aminobenzoate (Benzocaine), and how effective is recrystallisation as a purification technique for this compound?

Ameera Patel

B: 60 minutes (sample1)



R_f value for X = $\frac{X}{Y} = \frac{2.8}{5.6} =$ **0.5** ± 0.2

R_f value for Z = $\frac{Z}{Y} = \frac{4.1}{5.6} =$ **0.7** ± 0.2

Minimum Melting Point = **78.0°C** ± 0.3
Maximum Melting Point = **83.0°C** ± 0.3

Range of Melting = **5.0°C** ± 0.6

Fig. 7 TLC after recrystallisation of the sample created by a reflux time of 60 minutes

ANALYSIS

The theoretical yield of Benzocaine in grams that should be obtained from the synthesis can be calculated as follows:

1. The moles of PABA reacting in the esterification stage are calculated by dividing the mass added by the molar mass (3.00 g ÷ 137.136 g mol^{-1} = 0.022 mol)
2. The moles of ethanol reacting are calculated by multiplying the density of ethanol by the volume used and dividing this by the molar mass ((0.789 g cm^{-3} × 20.0 cm^3) ÷ 46.07 g mol^{-1}) = 0.343 mol.
3. We can now see that PABA is the limiting reagent since PABA and ethanol react in a one to one ratio: ($NH_2C_6H_4COOH + C_2H_5OH + 2H^+ \rightleftharpoons C_2H_5CO_2C_6H_4NH_3^+ + H_3O^+$). Hence the number of moles of PABA reacting equals the number of moles of Benzocaine produced.
4. The number of moles of PABA is multiplied by the molar mass of Benzocaine to give the theoretical yield (0.022 mol × 165.191 g mol^{-1} = 3.61g).

This can be summarised as:

$$\text{Theoretical Yield} = \left(\frac{3.00\text{ g}}{137.136\text{ g mol}^{-1}}\right) \times 165.191\text{ g mol}^{-1} = \mathbf{3.61\text{ g}}$$

The Percentage Yield obtained for each reflux time can be calculated by the following formula:

$$\text{Percentage Yield} = \frac{\text{Experimental Yield}}{\text{Theoretical Yield}} \times 100$$

For example, if the experimental yield obtained was 2.00g, the percentage yield would be:

$$\% \text{ Yield} = \frac{2.00}{3.61} \times 100 = 55.4\,\%$$

This table gives the following percentage yields of Benzocaine for the different reflux times.

	Reflux Time /min[20]	Mass of Benzocaine samples /g	Percentage Yield /%	Average mass of samples for each reflux time[21] /g	Percentage Yield based on average masses /%
1	30	1.78	49.3	1.78	49.3
2	45 (1)	2.24	62.1	2.12	58.7
3	45 (2)	2.00	55.4		
4	60 (1)	2.30	63.7	2.31	64.0
5	60 (2)	2.32	64.3		
6	75 (1)	2.22	61.5	2.25	62.3
7	75 (2)	2.28	63.2		
8	90 (1)	2.33	64.5	2.41	66.8
9	90 (2)	2.49	69.0		
10	105 (1)	2.14	59.3	2.26	62.6
11	105 (2)	2.38	65.9		
12	120	2.16	59.8	2.16	59.8

The percentage yields can now be plotted against reflux time to find the optimum reflux time. There are two possible interpretations of the data shown below as curve 1 and curve 2.

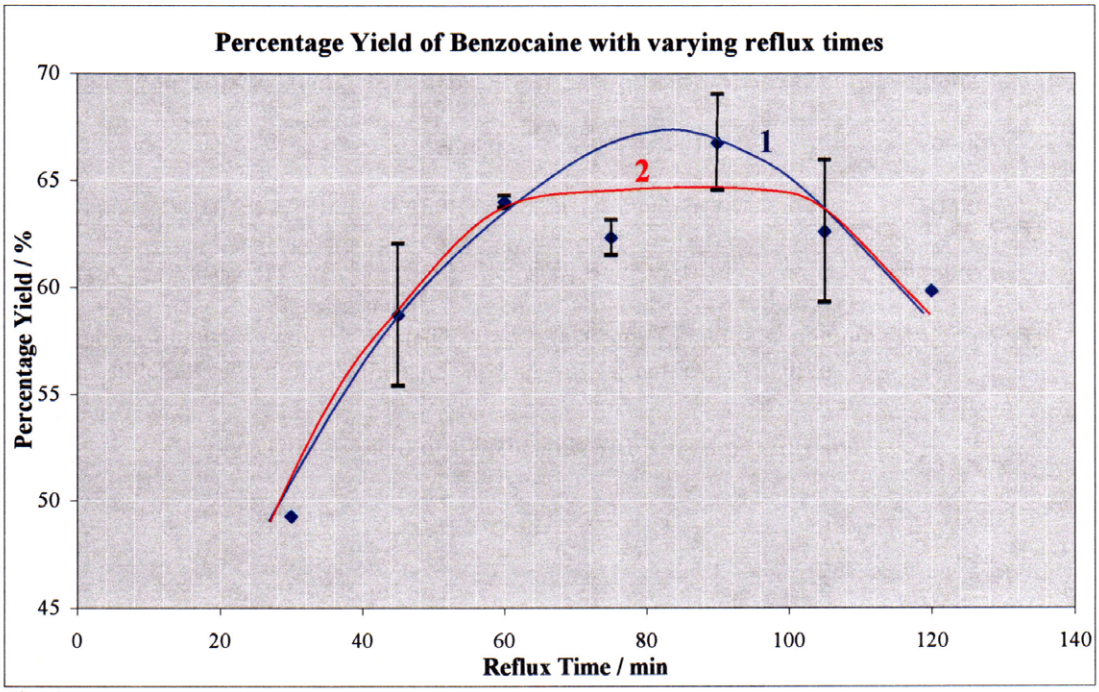


Fig. 8

[20] The numbers in brackets refer to repeat procedures producing different samples for the same reflux time.

[21] An average mass has been taken, so that there is one value for each reflux time, enabling a graph to be plotted.

How does reflux time affect the yield and purity of ethyl aminobenzoate (Benzocaine), and how effective is recrystallisation as a purification technique for this compound?

Ameera Patel

As a determinant of purity, the maximum melting point of each sample and the range over which the sample melts can be plotted against reflux time[22].

	Reflux Time /min	Maximum Melting Point /°C	Average Maximum Melting Point /°C	Range of Melting /°C	Average Range of Melting /°C
1	30	84.5	84.5	4.5	4.5
2	45 (1)	84.5	84.75	4.0	4.0
3	45 (2)	85.0		4.0	
4	60 (1)	85.0	85.0	3.5	3.5
5	60 (2)	85.0		3.5	
6	75 (1)	85.5	85.5	3.0	3.0
7	75 (2)	85.5		3.0	
8	90 (1)	86.0	86.25	2.5	2.5
9	90 (2)	86.5		2.5	
10	105 (1)	85.5	85.25	3.0	3.25
11	105 (2)	85.0		3.5	
12	120	85.0	85.0	3.5	3.5

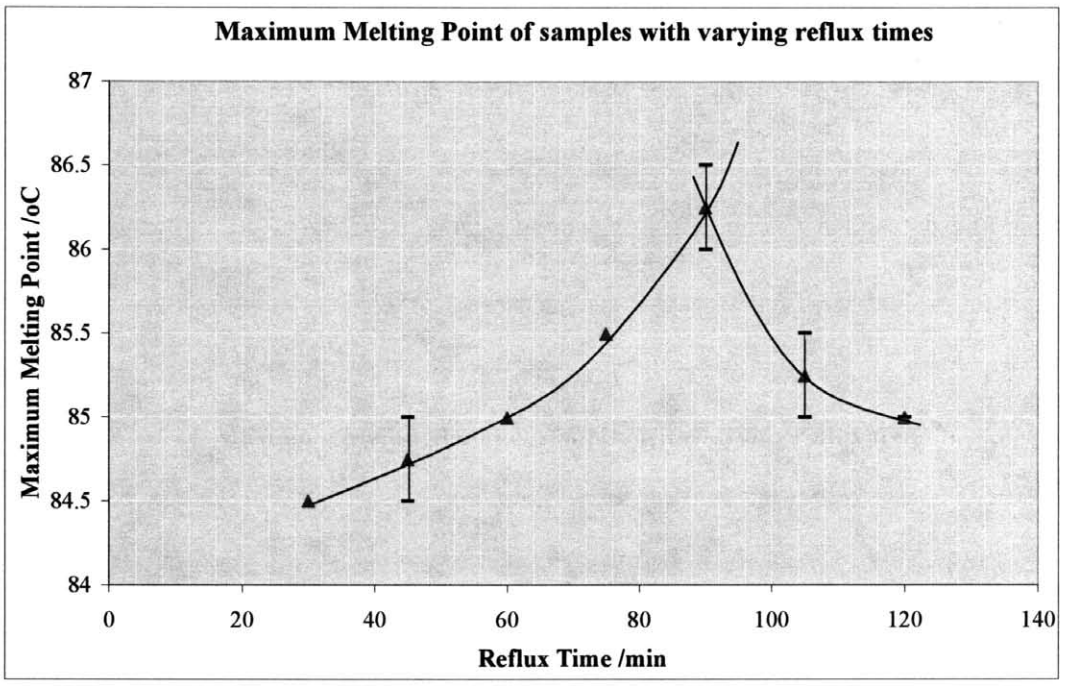


Fig. 9

The intersection of the two curves shows the reflux time that gives the highest maximum melting point. This reflux time is where the purest Benzocaine sample was obtained from the synthesis.

[22] The error bars show the smallest and largest value recorded from experimental data, and the points plotted are average values.

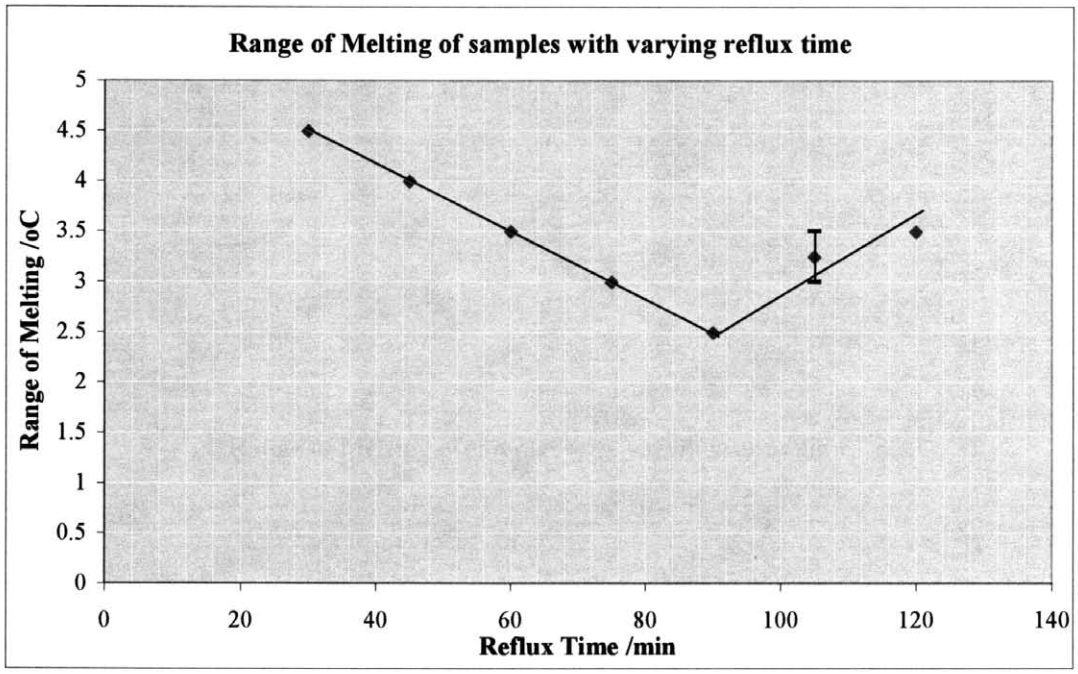


Fig. 10

Here, the same reflux time that gave the highest maximum melting point gave the smallest range. This can be considered the purest sample working on the assumption that the smallest range indicates the purest sample.

INTERPRETATION OF RESULTS

I will now explain the results and interpret the trends shown in the graphs in section 6. On observing the synthesis, I noticed that some reactants were residual in the reaction mixture after refluxing for shorted reflux times (30 minutes). However this disappeared with longer reflux times. Figure 8 shows that very short reflux times give a poor yield, whereas the optimum reflux time of 82-90 minutes gives the largest yield (from curve 1, as it is hard to decipher from curve 2).

Figure 8, as explained previously can be interpreted in two ways. Since the shapes of the curves are similar up to a reflux time of 60 minutes, I will discuss this first. Below a reflux time of 60 minutes, the reaction may not have gone to completion since it was moving towards equilibrium. Hence, some of the original reactants may have remained in the final product, unreacted. On the TLC plates for these reflux times, a secondary spot with an R_f value matching that of PABA appeared as an impurity. Theoretically, these spots should not have appeared as PABA dissolves in $NaOH_{(aq)}$ and would have passed through the Büchner funnel in solution. Hence, the residue of PABA may have been due to a greater number of moles of PABA present than $NaOH_{(aq)}$ or because of an inconsistency in stirring and in not washing the compound thoroughly enough. However, more generally, it is clear that the lower the reflux time, the greater the amount of original reactants present (from observations and accepted theory) and hence, the lower the yield as more PABA would be present for neutralisation and filtration as an aqueous salt. This resulted in the positive

gradient shown. Figures 9 & 10 show that in this range, an increase in reflux time resulted in an increase in maximum melting point and a decrease in range of melting. This fits with the explanation above in that an increase in reflux time causes a decrease in the amount of PABA present (more reactants react) and hence, the amount of impurity present (PABA) decreases.

From curve 1 in figure 8, it appears that an 82-90 minute reflux gives the optimum yield. However, the fact that this reflux time only produced a 65% yield can be attributed to spillages and the fact that in a reversible reaction, when equilibrium is reached, a 100% yield is not achieved. This reflux time explains the absence of impurities (secondary spots) on the TLC and why the maximum melting point obtained for this reflux time range (85.9-86.25 °C) was very close to pure Benzocaine (88 °C – Appendix II). The fact that the melting point indicated a slightly impure mixture would be expected at this stage since the compound has not been purified. External impurities from the apparatus or the surrounding environment may also have played a part.

I will now attempt to explain the latter part of figure 8. With curve 1, a reflux time is reached which gives the highest percentage yield, although it does not show an equilibrium being reached which would be expected for this reaction. After this point of highest percentage yield, longer reflux times cause the Benzocaine sample to decompose to its original reactants via a different pathway. This would explain why original reactants were present on the TLC plate for this reflux time, which increased with increasing reflux times. This would also explain why the optimum reflux time for Benzocaine synthesis is quoted at 75 minutes[23] (as longer reflux times cause a decomposition). This explanation suggesting that Benzocaine is unstable for long periods of heating may explain the findings with recrystallisation. The problem with this interpretation is that the yield obtained for a reflux time of 75 minutes does not fit the shape of the graph (although it could be an anomaly). This suggests that curve 2 may provide a better explanation. Curve 2 shows the accepted idea of a reaction mixture moving towards equilibrium (the plateau). The maximum yield is therefore obtained for reflux times greater than or equal to 60 minutes although there is a downward slope for high reflux times showing decomposition. The problem with this theory is that a definitive optimum reflux time is not reached (although one has been quoted) and this trend does not fit with the melting point data. Despite this, I think that curve 2 provides a better interpretation since it includes the idea of equilibrium and still fits with the data for higher reflux times (decomposition). If this is the case, any reflux time in the plateau region (60-100 minutes reflux) could be quoted as an optimum reflux time, and this range can be narrowed down to 82-90 minutes using the melting point data (figures 9 & 10) because this range gives the highest purity. However, further work needs to be carried out.

With recrystallisation, the samples obtained had melting points and R_f values considerably lower than that of Benzocaine. With the sample produced from a 60-minute reflux after recrystallisation, one small spot on the TLC had an R_f value the same as that of Benzocaine. However, this represented the small quantity of Benzocaine sample that had not been affected by the process. Considering the main spots on the TLC, it appears that recrystallisation caused the Benzocaine sample to decompose. The conclusion given above suggests that Benzocaine becomes unstable for long periods of heating. This may also be the case at very high temperatures. The other possibility is that a side reaction occurred to produce a different compound (although an exact reaction cannot be specified). Although no impure spots were seen on the TLC plates, the large range over which the samples melted indicates that a number of impurities were present. These may not have shown up

[23] McMaster Univeristy, Ontario. http://www.chemistry.mcmaster.ca/~chem2o6/labmanual/expt9/2o6exp9.html [17 August 2006]

on the TLC if they were too soluble or insoluble in the solvent, or if they were present in very small quantities.

CONCLUSION:

From my results, it appears that the optimum reflux time[24] is between 60 and 100 minutes (since an equilibrium was reached for this period). The melting point data narrows this to 82-90 minutes. However, to determine this figure more accurately, further work needs to be carried out firstly to determine with a greater degree of accuracy the shape of the graph in figure 8. A 90-minute reflux gave the best purity. Reflux times below 82-90 minutes gave lower yields and lower purities. For very high reflux times, a decomposition occurred, resulting in decreased purity. Recrystallisation does not appear to improve purity in this case, and further investigation is required to determine the composition of the recrystallised samples and whether other solvents would give better results; in any case, it seems likely that something unexpected occurred since the same trend was observed with both recrystallised samples.

EVALUATION

8.1 Random Error

The quantities and volumes of reactants used are subject to a possible random error. The sulphuric acid and ethanol are subject to random errors of ± 0.5 cm^3 from the measuring cylinders and the PABA is subject to a random error of ± 0.005 g. However, since a number of the repeat readings were more than 10% from each other, the random error is not a major factor in explaining variation.

8.2 Systematic Error

The method used for the synthesis was in some ways limited. Firstly, it was difficult to control the reflux times accurately; after the heater was switched off, the reaction mixture continued to boil for a number of minutes. Hence, consistency between repeats was compromised. In all instances, the mass of the dry sample cannot be considered very accurate (too small) due to residue in the apparatus[25] and spillage after drying. Since this error was not consistent throughout the procedure, it may have led to inaccuracies between results. These significant errors led to large differences in repeat readings in some cases, and since there was not enough time to take more repeat readings, it became difficult to discern which results were anomalous. In figure 8, the dry mass of sample with a reflux time of 75 minutes appears too small (especially as this reflux time is quoted to give the largest yield). However, the fact that the repeat readings for this reflux time are so close together (2.6%) raises questions about the accuracy of the procedure, as does the fact that there is a difference between the literature value and my value for the optimum reflux time.

Another point is that on chilling the reaction mixture after esterification, some samples were found to contain crystallised lumps. As a result, the quantity of $NaOH_{(aq)}$ added is unlikely to have been inaccurate since this solid did not disintegrate in most samples. I tested the pH of this residue and found it to be acidic. This is likely to have affected the mass and purity of the sample. Further tests

[24] The reflux time that gives the largest yield
[25] This may account for the low percentage yields obtained

may be required to determine what occurred. A possibility is that some of the chemicals may have become contaminated, as this solid was not observed in preliminary experiments.

With TLC, the components of the solvent were volatile (by different amounts) so it was difficult to keep the ratio of components constant even though the solvent was covered. These inconsistencies can be seen in the R_f values for the same compound, PABA, (see footnote 19). This may have affected the R_f values for the recrystallised samples more as they were calculated over a longer time frame. Another point is that the R_f value of commercial Benzocaine was never obtained for the solvent used. It is therefore incorrect to assume that the primary spot on the TLC plates for the samples was Benzocaine although this seems likely from the melting points obtained.

Finally, the fact that the graph in figure 9 comprises of two curves whilst the shape of the graph in figure 10 comprises of two straight lines is unexplained. Further investigation is required to determine whether these graphs are correct, or whether the difference is a result of experimental error. Taking repeat readings and averages of only accurate data as opposed to all data would ensure greater accuracy of results and hence would enable a more accurate line of best fit to be drawn through the points in figure 8. Also, the apparatus used was slightly limiting, for example, it was difficult to determine the exact neutralisation point in the second stage of the synthesis. Hence, my conclusion cannot be fully verified, as enough data was not collected. However, my conclusion does appear to fit with accepted theory, and whereas the exact numerical details (for example the optimum reflux time) may not be exactly clear due to limitations of the procedure, part of my explanation for figure 8 (the former part with a positive gradient and the idea of equilibrium) is likely to be correct since the error bars do not overlap for the former part of the graph, and the observed trend was as predicted.

8.3 Evaluation of sources

Since books are generally more reliable than web pages, the book used to investigate techniques was probably more reliable that the web pages used. However, the uncertainty in using web data was reduced by finding the contents of a web page on at least one other independent site where possible. For example, the mechanism for esterification was searched for on two other websites before the contents of the original website were deemed trustworthy. Since this was not always possible, the reliability of some statements is questionable.

Ameera Patel

UNANSWERED QUESTIONS

Unfortunately, not all questions could be answered within this experiment. It would be interesting to further investigate, firstly, the trends shown by percentage yields and melting points by increasing the range and frequency of reflux times and repeating the results for accuracy. This would also be able to clarify the progression of the reaction in figure 8.

The fact that the reflux time producing the best yield showed no impurities on the TLC but a melting point slightly lower than that of commercial Benzocaine is interesting. High-pressure liquid chromatography (HPLC) may disclose whether these impurities came from the experiment (reactants in very small quantities) or whether they came from external sources, and if so, what the impurities were.

The fact that recrystallisation caused the sample either to decompose or to react is very interesting since it should have purified the sample. Since the range of melting was so large, many compounds may have been present. Finding the contents of the sample (again using HPLC) may indicate the pathway by which the Benzocaine samples either decomposed or reacted.

Finally, why a hard, solid precipitate formed in some samples when cooling the reaction after the first stage is puzzling. Again, further tests on this (for example HPLC) may reveal the composition of the solidified mixture and other investigative work may disclose why it formed in certain samples: whether it was a result of the reflux time used or contamination.

The use of such advanced equipment as suggested in this section exceeds the possibilities provided in a school laboratory. I would very much like to continue this research at university where such modern analytical tools are available.

Word count = 3977

How does reflux time affect the yield and purity of ethyl aminobenzoate (Benzocaine), and how effective is recrystallisation as a purification technique for this compound?

Ameera Patel

BIBLIOGRAPHY

Answers Corporations. *Fischer Esterification.* 2006.
http://www.answers.com/fischer%20esterification [1 August 2006]

Arokor Holdings Inc (Chemical Land21). *Chemical Search.* 2000.
http://www.chemicalland21.com/index.html [5 August 2006]

Blackwell Publishing Group. *Organic Chemistry II: Fischer Esterification Mechanism.*
http://www.blackwellpublishing.com/11thhour/book6/oc2ch7.html [2 August 2006]

Clark, J. *ChemGuide, The mechanism for the esterification reaction.* 2004.
http://www.chemguide.co.uk/physical/catalysis/esterify.html [2 August 2006]

European Agency for the Evaluation of Medicinal Products (EMEA). *Committee for Veterinary Medicinal Products: Benzocaine.* November 2001.
http://www.emea.eu.int/pdfs/vet/mrls/081101en.pdf [31 July 2006]

InfoChem (Spresi Web). *Organic Chemistry Portal: Fischer Esterification.* 2002-2006.
http://www.organic-chemistry.org/frames.htm?http://www.organic-chemistry.org/namedreactions/fischer-esterification.shtm [1August 2006]

LaborLawTalk Dictionary. *Benzocaine.*
http://dictionary.laborlawtalk.com/benzocaine [31 July 2006]

MacMillan, J.G. *Mechanism of Fischer Esterification.* 27 July 2001.
http://www.chem.uni.edu/~macmilla/mcmurry/mcmurry_chapter_21/sld017.htm [2 August 2006]

McMaster University, Ontario, Canada. *Chem 206, Laboratory Manual, Reactions of Carboxylic Acids and their Derivatives.* 1997-1998.
http://www.chemistry.mcmaster.ca/~chem2o6/labmanual/expt9/2o6exp9.html [17 August 2006]

Merck KgaA Pharmaceuticals Damstadt, Germany. *ChemDat Chemicals Database.* 31 August 2006.
http://uk.chemdat.info/mda/uk/ [5 August 2006]

Miller, G.A . Wordnet, University of Princeton. *Ethyl Aminobenzoate.*
http://wordnet.princeton.edu/perl/webwn?s=benzocaine [31 July 2006]

Pennsylvania State University, Pennsylvania. *The Preparation of the local anaesthetic, Benzocaine, by an esterification reaction.* 27 June 2006.
http://courses.chem.psu.edu/chem36/Web%20Syn06/Exp86Syn06.pdf [31 July 2006]

Prof. S Ahmed, University of Kingston, Personal Correspondence [20 June 2006]

Sharp,J.T. Gosney,I. Rowley, A.G. *Practical Organic Chemistry (A student handbook of techniques)*, London, Chapman & Hall 1989.

Ameera Patel

APPENDIX I

1. APPARATUS

For the investigation, four types of apparatus are required for the four procedures outlined in the previous section.

For the synthesis of Benzocaine:

- Round bottom flask [quickfit], 250cm^3 (x1)
- Reflux Condenser [quickfit] (x1)
- Heating Mantle (x1)
- Clamp Stand (x2)
- Measuring cylinder, 25cm^3 (x1)
- Measuring cylinder, 10cm^3 (x1)
- Weighing boat (x7)
- Metal Spatula (x1)
- Plastic container, 1dm^3 (x2)
- Stirring rod (x1)
- pH paper
- Ice
- Beaker, 1dm^3 (x1)
- Büchner funnel & volumetric flask with vacuum pump (x1)
- Filter Paper (x7)
- Watch glass (x7)
- Stopwatch (x1)
- Oven at <70°C

For Melting Point determination:

- Melting Point Apparatus
- Capillary tubes
- Bunsen Burner (x1)
- Matches
- Heat Proof Mat (x1)
- Thermometer, max. 100°C (x1)

For Thin Layer Chromatography:

- TLC Plates (x7)
- Beaker, 100 cm^3 (x1)
- Capillary tubes
- Bunsen Burner
- Heat Proof Mat
- Matches
- Aluminium foil
- Pencil & Ruler
- Hairdryer
- Low Intensity UV lamp

For Recrystallisation:

- Beaker, 250 cm^3 (x1)
- Stirring Rod (x1)
- Electric Heater (x1)
- Büchner funnel and volumetric flask under vacuum (x1)
- Filter Paper (x2)
- Watch Glass (x1)
- Oven at < 70°C

The apparatus listed above does not list quantities required for repeat readings.

How does reflux time affect the yield and purity of ethyl aminobenzoate (Benzocaine), and how effective is recrystallisation as a purification technique for this compound?

Ameera Patel

Drawing of apparatus used for esterification

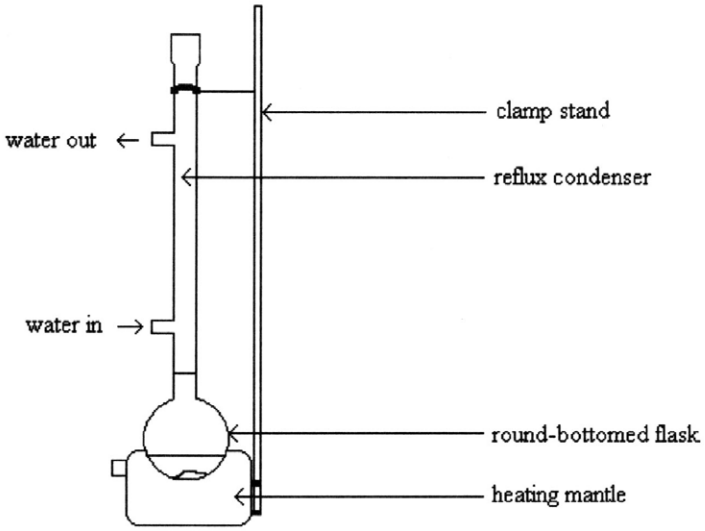


Fig. 11 Esterification apparatus

2. REAGENTS / CHEMICALS

For the synthesis of Benzocaine:

- Concentrated Sulphuric Acid (c.$H_2SO_{4(aq)}$)
- Ethanol ($C_2H_5OH_{(l)}$), liquid
- 4-aminobenzoic acid ($C_7H_7NO_{2(s)}$), solid
- Distilled water
- Sodium Hydroxide solution ($NaOH_{(aq)}$), 1M

For Thin Layer Chromatography:

- 1-chlorobutane ($C_3H_7CH_2Cl_{(l)}$)
- Petroleum Ether, liquid
- Propanone ($CH_3COCH_{3(l)}$)

For Recrystallisation:

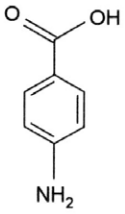
- Petroleum Ether, liquid
- Propanone ($CH_3COCH_{3(l)}$)

Ameera Patel

APPENDIX II

Information on Reagents and Products[26]

1. 4-aminobenzoic acid



Molar Mass: 137.136 g/mol

Melting Point: 187 °C

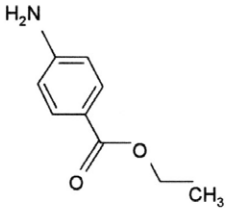
2. Ethanol

Molar Mass: 45.7 g/mol

Melting Point: -114.3 °C

Density: 0.789 g/cm³

3. Ethyl Aminobenzoate



Molar Mass: 165.191 g/mol

Melting Point: 88 °C

[26] Merck KgaA Pharmaceuticals Damstadt, Germany. http://uk.chemdat.info/mda/uk/ [5 August 2006]
Arokor Holdings Inc. Chemical Land21. http://www.chemicalland21.com/index.html [5 August 2006]