E29 Preparation of Aspirin (Acetylsalicylic Acid) and Thin-Layer Chromatography of Analgesic Drugs

THE TASK

To prepare a pharmaceutical compound and test its purity.

THE SKILLS

By the end of the experiment you should be able to:

- use recrystallisation to purify solids,
- load, prepare and develop thin layer chromatography plates,
- use thin layer chromatography to test purity and to identify the components of mixtures.

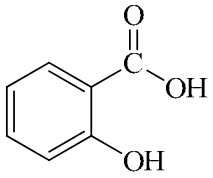
OTHER OUTCOMES

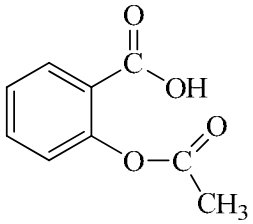
- You will develop an appreciation of the techniques used in organic and pharmaceutical science to synthesise pure organic compounds and to identify them.
- You will develop an understanding of how thin layer chromatography is used to test sample purity.

INTRODUCTION

Salicin, a β-glycoside of salicylic acid, present in the leaves and bark of willow trees (genus *Salix*), has been used for centuries in a variety of herbal remedies. *In vivo*, it is converted into salicylic acid which acts to reduce inflammation and lower the temperature of patients suffering from fever.

Salicylic acid itself is unsuitable as a drug since large doses have an unpleasant taste and also cause gastric irritation. These problems were largely overcome by the introduction of acetylsalicylic acid or Aspirin® by the German company Bayer in 1899. The name "aspirin" is derived from *a*cetylated *spir*aeic acid (the old name for salicylic acid). Aspirin is an ester of salicylic acid which passes through the stomach unchanged before being hydrolysed by the basic medium of the intestine to form the active compound.

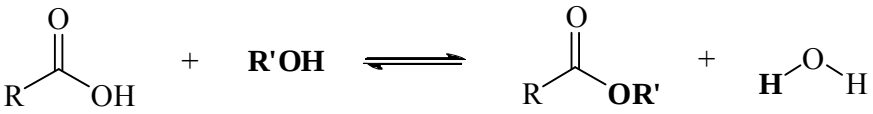
salicylic acid 

acetylsalicylic acid 

Aspirin acts to inhibit the production of prostaglandins, which are produced in all parts of the body and which have important functions in pain sensation, inflammation and swelling. Aspirin is taken in low doses to reduce the risk of strokes in people who have risk factors such as hypertension. Aspirin can cause stomach upsets and allergies and in some young people can trigger the potentially fatal Reye's syndrome.

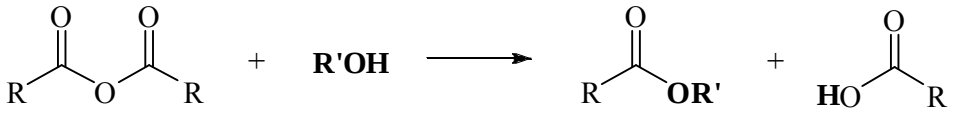
Esterification

When an alcohol or phenol reacts with a carboxylic acid, the products are water and an ester. The esterification reaction is slow and as soon as the products begin to form, the reverse reaction, hydrolysis, begins. An equilibrium is finally attained with all reactants and products present.



At 20 °C the rate of reaction in both the forward and the backward directions is slow and it takes many days to attain equilibrium. Heating increases the rate of both the forward and reverse reactions, thus achieving the equilibrium state much faster, but it does not significantly alter the position of equilibrium. The value of the equilibrium constant, *K*, at room temperature is typically about 10 (*i.e.* the reaction does not go to completion), so the yield of the desired product will not be very high.

An alternative method for the preparation of esters is to treat the alcohol with a reactive carboxylic acid derivative, for example a carboxylic acid anhydride. These reactions are effectively irreversible. They are also rapid, particularly when catalysed by strong acids.



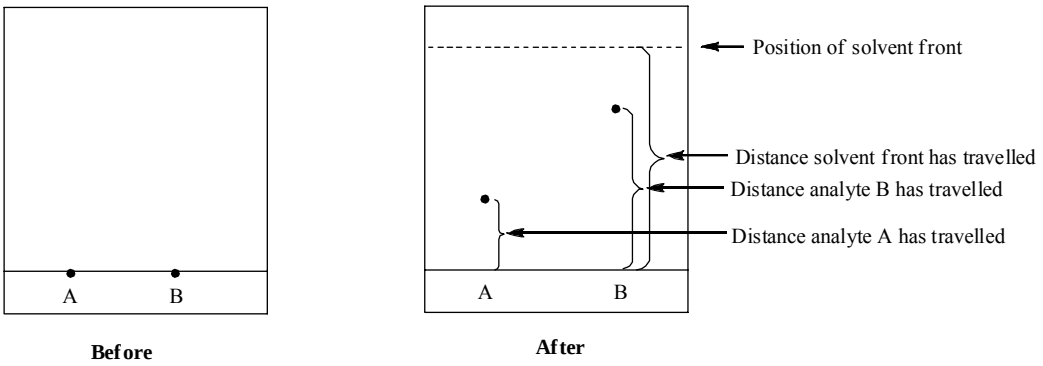
acid anhydride alcohol ester carboxylic acid

Thin-layer chromatography

Thin-layer chromatography (TLC) is an important technique in organic chemistry. TLC can be used to assess the course of a reaction, to assess the purity of a sample and also to identify unknown compounds by comparison with standards. TLC is used in this experiment to identify the components of analgesic (pain-killing) tablets as well as confirming the identity and purity of the aspirin synthesised.

In TLC, a thin-layer of *stationary phase* is applied to a carrier plate. The plates supplied are prepared commercially and consist of a thin layer of silica on an aluminium carrier plate. The developing liquid or *mobile phase* travels up the plate by capillary action and the compounds being analysed are carried along with it at varying speeds depending upon the strength of their attraction to the stationary phase and the polarity of the mobile phase.

The following diagram illustrates the appearance of a TLC plate before and after development:



Analyte A is more strongly attracted to the stationary phase than analyte B. The position of the analyte spot is defined by its retention factor, R_f.

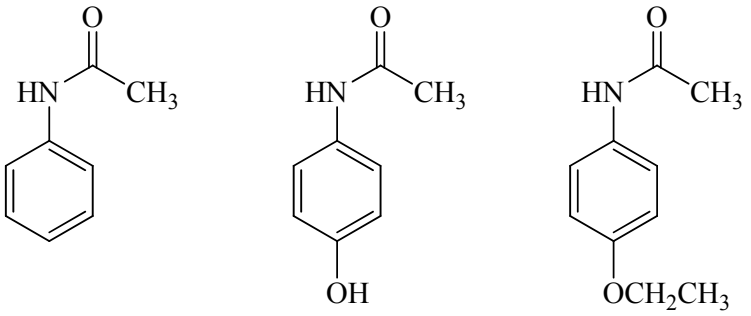
R_f = distance analyte has travelled / distance solvent front has travelled

Since many analytes are colourless it is necessary to treat the TLC plate so that the spots can be seen. This can be achieved in a number of ways including, as in this experiment, the use of UV light or iodine vapour. Different analytes can be distinguished by their R_f values as well as by their appearance under UV light or after treatment with other visualising reagents.

This experiment uses TLC to analyse standards of caffeine and three analgesics, acetylsalicylic acid (aspirin), acetaminophen (paracetamol) and ibuprofen. You will then attempt to identify the active ingredient(s) of a commercial tablet by comparison with these standards.

Other analgesics

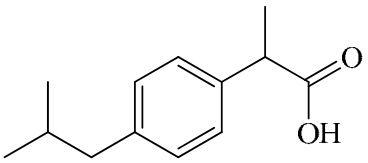
Some acylated aromatic amines that have been used as pain-killers, include acetanilide, acetaminophen and phenacetin.



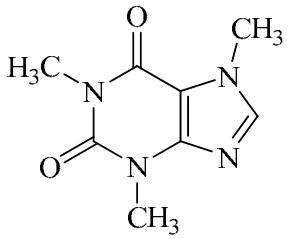
acetanilide | acetaminophen (paracetamol) | phenacetin

Phenacetin is now known to be carcinogenic as well as having other undesirable side-effects and was withdrawn in the late 1960s after almost a century of use. The only one of these compounds still in general use is acetaminophen (paracetamol, Panadol®). It is the analgesic of choice for people who are allergic to aspirin, although prolonged use can cause kidney damage and overdoses can cause fatal liver damage.

Aspirin and acetaminophen are active ingredients in different commercial pain-killers and in "cold cures". In addition to these there may be other active ingredients such as ibuprofen, caffeine and inert ingredients including binders such as starch or carboxymethylcellulose.



ibuprofen



caffeine

SAFETY

Materials	Nature
salicylic acid	
acetic anhydride	
sulfuric acid	
hexane	
acetic acid	
ethyl acetate	
ethanol	
methanol	
aspirin	
acetaminophen	
ibuprofen	
caffeine	

Indicate, by signing, that you have understood the information in the safety table.	I understand the safety information	Demonstrator's Initials

LAB-WORK

		20

Safety Note. Many of the liquids used in this experiment are quite volatile. All organic residues must be placed in the appropriate containers in the fume hood.
Acetic anhydride and sulfuric acid can cause serious burns on contact with skin. In case of contact with either, wash the skin thoroughly with soap and water and seek advice from a demonstrator. Avoid breathing acetic anhydride vapours. Wash any spillage from the desk top immediately. Do NOT pour acetic anhydride down the drain.
The aspirin you will prepare in this experiment is relatively impure and MUST NOT be consumed.
Dispose of any excess solid chemical in the appropriate container in the front fume hood.

Experiment 1: Synthesis of acetylsalicylic acid (aspirin)

Acetic anhydride and salicylic acid react to produce acetylsalicylic acid and acetic acid; sulfuric acid is used as a catalyst. The excess acetic anhydride is then decomposed with water to form acetic acid. Acetylsalicylic acid is not very soluble in cold water (~ 0.25 g per 100 mL) and consequently it can be isolated by diluting the reaction mixture with water and filtering off the solid product.

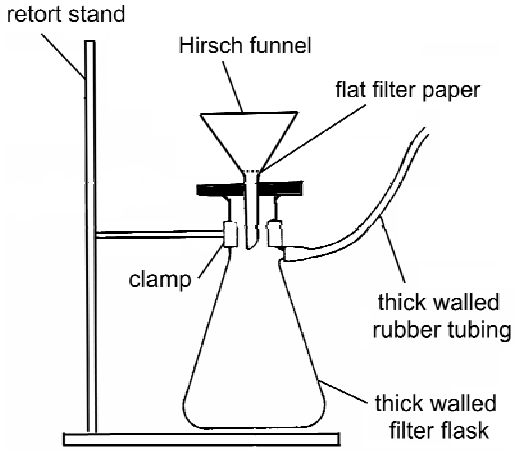
Weigh out salicylic acid (3.0 g ± 0.1 g) in a clean, dry, 100 mL conical flask. Record the mass (to 2 decimal places).

Mass of salicylic acid _____ g

Working in a fume hood, take acetic anhydride (5.0 mL) in a clean, dry, 10 mL measuring cylinder and pour it into the flask containing the salicylic acid in such a way as to wash down any crystals that may have adhered to the walls of the flask. While swirling the flask, carefully add 3 drops of concentrated sulfuric acid. (Caution! Corrosive – avoid contact with skin and clothing.) Cover the flask with a small watch glass to prevent condensation of water inside the flask during heating on the steam bath. Heat the flask on a steam bath for 15 minutes. Move onto Experiment 2 while you are waiting.

After heating for 15 minutes, remove the flask from the steam bath and while the contents are still hot, **cautiously** add (ONE DROP AT A TIME, USING A PIPETTE) water (5 mL). After the reaction subsides, add ice-water[1] (35 mL) all at once and swirl the flask for a few seconds. Remove the flask from the fume hood and chill it in an ice-bath[2]. If crystallisation is slow it may be helpful to scratch the inside of the flask with a stirring rod. While you wait for crystallisation to be complete, set up your filtration apparatus for the next step and ensure there is a good seal between the funnel and the filter flask[3].

After crystallisation is complete, decant the liquid from the flask into the Hirsch funnel. Minimise transfer of solid, but if you inadvertently transfer some, it's OK. Break up any lumps of solid in the flask using a stirring rod and add ice-water (10 mL). Swirl the flask, chill briefly and empty all the contents of the flask into the Hirsch funnel. Use an additional 10 mL of chilled water to transfer any solid which may be left in the flask. Run air through the product for 5 minutes to help dry it out. You may like to increase the vacuum to accelerate the drying process[4]. If necessary, dry the product further by squeezing it between two pieces of filter paper with a beaker.



[1] Ice-water is prepared by chilling water in a flask on an ice-bath. It does not contain ice.
[2] An ice-bath is a mixture of ice and water. The contact between cold water and a flask is much greater than that between ice and a flask allowing for greater conduction of heat. Use a 400 mL beaker to hold your ice-bath.
[3] Test the vacuum by applying the end of the rubber tubing to your thumb. The vacuum should just be able to hold the tube onto your thumb. If you use a stronger vacuum you may rupture the filter paper and will have to set up the filtration apparatus again.
[4] The flow of air past the sample due to the vacuum will help dry your product.

Transfer the solid to a clean, dry, pre-weighed 100 mL beaker. Determine the mass of the product.

Mass of crude aspirin		g

Warning! Your product is crude compared with commercial Aspirin. Do NOT ingest it.

What was the purpose of adding the sulfuric acid?

Why was it important to prevent condensation from entering the flask during the reaction? What was the purpose of adding water carefully at the end of the reaction? Write an equation for the reaction involving the water.

Why did you chill the flask on ice before filtering to collect the solid?

Demonstrator's Initials	

Experiment 2: Thin Layer Chromatography

A number of analgesics are examined using thin layer chromatography (TLC) and the pain killer in an unknown tablet is identified. The usefulness and limitations of two solvent systems are investigated by testing solvent combinations with different polarities.

Preparation of the solvent tanks

Prepare and label two solvent tanks (250 mL beakers) each containing about 10 mL of the two pre-mixed solvent combinations provided.

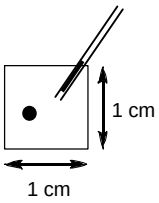
Solvent A is a 65:30:5 mixture of hexane, ethyl acetate and acetic acid respectively.

Solvent B is a 45:50:5 mixture of hexane, ethyl acetate and acetic acid respectively.

Cover each tank with a watch glass so that the atmosphere of the tank becomes saturated with the solvent vapour. About 0.5 cm of solvent should cover the base of the tank.

CAUTION: DO NOT INHALE THE SOLVENT OR ALLOW IT TO CONTACT YOUR SKIN

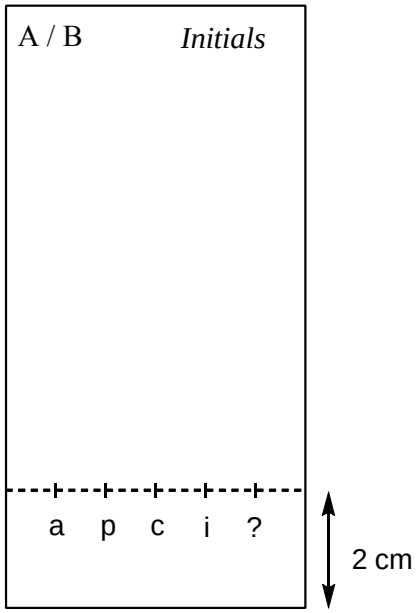
Practise loading the TLC plates



To load the samples onto your plate you will use a plastic pipette tip as a capillary. Practise spotting on a small practice plate (1 cm × 1 cm). Dip the capillary into one of your samples. Briefly touch the tip of the capillary onto the plate. The solvent will evaporate in a few seconds. Reapply the tip of the capillary onto the same spot on the plate. Try to keep the spots as small as possible. You will need to spot your analyte 3 times. Run out any residual analyte by touching the capillary tip onto a piece of paper towel.

Preparation of the TLC plate

Handle TLC plates by the edges only, taking care not to touch the white surface with your fingers. Collect two TLC plates of dimensions 10 cm × 5 cm. Place the plates on a clean dry surface and using a pencil and a ruler draw a line 2.0 cm from the short edge. Press lightly with the pencil so as not to damage the silica layer. On this line, mark points starting 0.9 cm in from one side and then at 0.8 cm intervals to give 5 points. One point is for the unknown and the others are for the standards. Label the points lightly with pencil and include your initials at the top of the plates. Label one plate A and the other plate B.



Collecting the unknown

Your demonstrator will have several unknowns already in solution. You are asked to identify one of them, but can try more than one if you wish. Results will be shared in a group discussion.

Loading the TLC plates

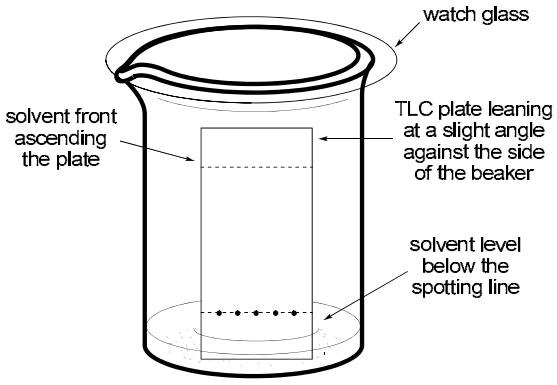
The analytes you will use in this experiment are:

1) a : aspirin (acetylsalicylic acid)
2) p : paracetamol (acetaminophen)
3) c : caffeine
4) i : ibuprofen
5) ? : one unknown.

Once you are comfortable with the spotting technique, spot each analyte onto the appropriate mark on your TLC plates. Use the same technique as practised above: spot each analyte three times, allowing the first spot to dry before adding the next.

Developing the TLC plates

When the spots are dry, place the plate into the solvent tank using a wooden peg to grip the plate at the very top. Make sure that the solvent level is below the baseline. Put the cover back on the tank. Allow the plate to develop until the solvent front is about 1 cm from the top (about 15 minutes). Move onto Experiment 3 while you are waiting. Use a wooden peg to grasp the plate above the level of the solvent and remove it from the solvent tank. Immediately mark the position of the solvent front with pencil. Place the plate on some paper towel in the fume hood until it is dry (about 1 minute).



**CAUTION: DO NOT REMOVE WET PLATES FROM THE FUME HOOD;
DO NOT LEAN INTO THE FUME HOOD TO MARK YOUR PLATE.**

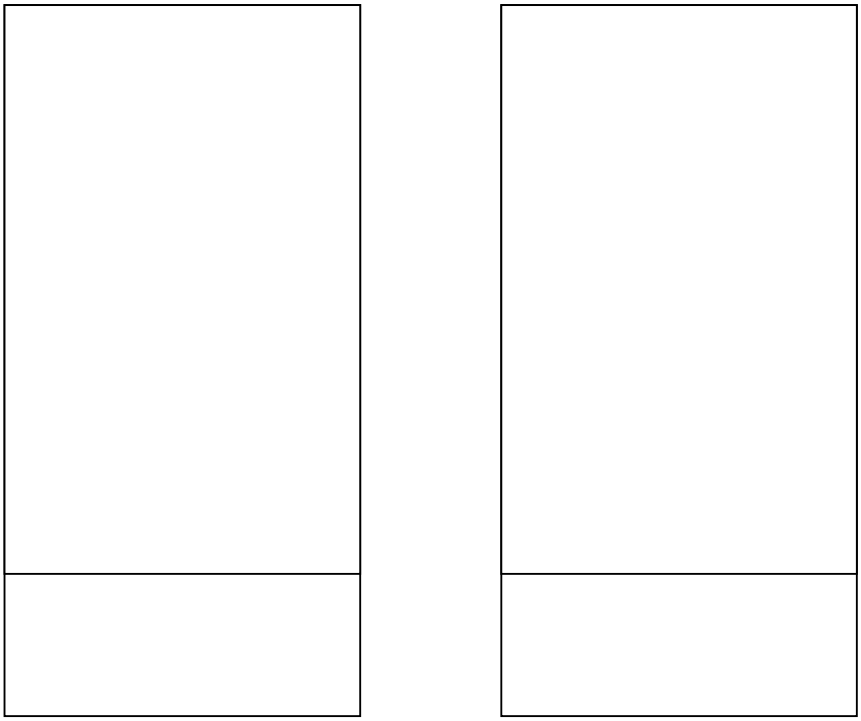
Remove the dry TLC plate from the fume hood and rule a pencil line to show the solvent front.

Visualisation of the spots

CAUTION: UV LIGHT IS HARMFUL TO EYES AND SKIN. DO NOT LOOK DIRECTLY AT THE LIGHT OR PLACE YOUR HANDS UNDER THE LIGHT.

Using a large wooden test-tube holder, place the plate under the UV light. Using a long pencil, lightly outline the shape of the spots on the plate. Note the different colours of the spots. Calculate the R_f values of the spots for solvents A and B. Note that some of the ingredients in the commercial tablet may cause streaking. Streaking can also be caused by loading too much analyte onto the plate. (See TLC Troubleshooting on page E29-14, which shows what the TLC plate should look like and, if it doesn't, how to fix the problem.)

Sketch your TLC plates below. Indicate which spots are visible under long wave-length UV light ($\lambda = 365$ nm) and which spots are visible under short wave-length UV light ($\lambda = 254$ nm).



Solvent system A Solvent system B

Using a wooden peg place the plate in the iodine vapour chamber (a beaker with some solid iodine in it and a watch glass on top) and leave for a few minutes. Observe any colour changes and indicate these on your sketches. Remove the plate from the chamber using a wooden peg and place it on some paper towel in the fume hood for 5 minutes. Identify your unknown by completing the following table and answer the questions below. Dispose of your TLC plate in the appropriate container in the front fume hood.

Solvent A	aspirin	paracetamol	caffeine	ibuprofen	unknown
Colour (long λ UV)					
Colour (short λ UV)					
Colour (I_2)					
R_f					

Solvent B	aspirin	paracetamol	caffeine	ibuprofen	unknown
Colour (long λ UV)					
Colour (short λ UV)					
Colour (I_2)					
R_f					

Silica is polar and the mobile phase is less polar. Which is the most polar analyte of those tested?

Comment on the usefulness of each solvent in separating the analytes.

Why is the TLC plate marked with a pencil and not a pen? (Note: This is the same reason that any labels on sample vials should be written in pencil.)

Demonstrator's Initials	

Experiment 3: Recrystallisation of Aspirin

The basic principles employed in recrystallisation are:

- dissolution of the crude material in the smallest amount of a suitable hot solvent;
- filtration while hot to remove any insoluble impurities (if necessary);
- cooling of the solution so that the desired compound, which makes up the majority of the sample, preferentially crystallises;
- the soluble impurities are left dissolved in the solution (known as the "mother liquor");
- filtration to separate the recrystallised solid from the mother liquor.

A range of solvents can be used for the recrystallisation of aspirin, but one of the best and cheapest is a 5 %(v/v) ethanol/water mixture. Prepare this solvent by combining water (95 mL) and ethanol (5 mL) in a 250 mL conical flask. Add two anti-bumping granules and place the flask on a steam bath to heat.

The volume of hot solvent required in a recrystallisation depends on the amount of crude sample and its solubility in the solvent. In this recrystallisation, approximately 20 mL of the solvent per gram of crude aspirin will be needed. What was the mass of your sample? What volume of solvent do you expect to need? Check your answer with a demonstrator.

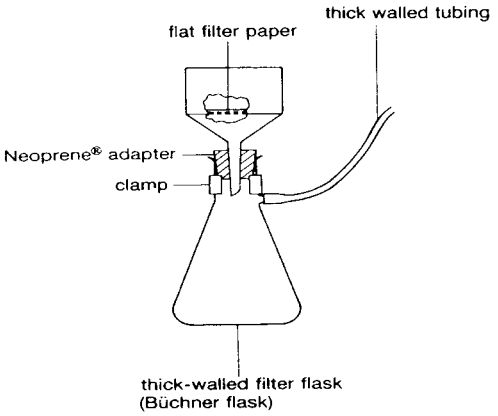
Mass of crude aspirin	g
Expected volume of solvent	mL

Set aside about 5 mg of your crude aspirin sample in a semi-micro test tube and place the rest in a clean 100 mL conical flask and add two boiling chips.

Once the water/ethanol mixture is hot (small bubbles are forming) add your estimated volume to the aspirin sample. Swirl the flask to mix the contents and warm it on the steam bath. (Caution! The flask is hot!) Keep the solvent hot on the steam bath throughout this process. If any solid remains, add hot solvent in 5-10 mL portions, with warming between each addition, until all the solid has dissolved.

Once all the solid has dissolved, place the flask on a heat-proof mat on your bench to cool. Crystals should soon begin to deposit in your flask. When the solution has cooled to about 40 °C (*i.e. warm*, not hot, if held in your hand) place the flask in an ice-bath (use a 500 mL beaker) and allow it to cool for 5-10 minutes to complete crystallisation.

Set up a vacuum filtration apparatus using a Büchner funnel, making sure the filter flask is clean. Dampen the filter paper circle with a little water, apply the vacuum and check that a good seal is obtained. Collect the crystals by swirling the flask to suspend them and then pouring the suspension into the filter funnel, trying to leave as few crystals in the conical flask as possible. Any remaining crystals can be transferred by reusing some of the cold filtrate (recycling the filtrate) – DO NOT use fresh solvent for this procedure.



Dry the crystals by drawing air through the product for 5 minutes. The vacuum can be increased to accelerate the drying process. If required, the base of a small beaker can be used to squash the crystals between two filter papers to remove any further solvent. Consult a demonstrator to see if this is necessary. Transfer the crystals to a labelled, pre-weighed, clean 100 mL beaker. Determine the mass of recrystallised aspirin.

Mass of recrystallised aspirin _____ g

What is the theoretical yield (maximum amount of aspirin you could have made) for this experiment?

Calculate your percentage yield of recrystallised aspirin.

Why can you recycle the filtrate to transfer crystals, but not use fresh solvent?

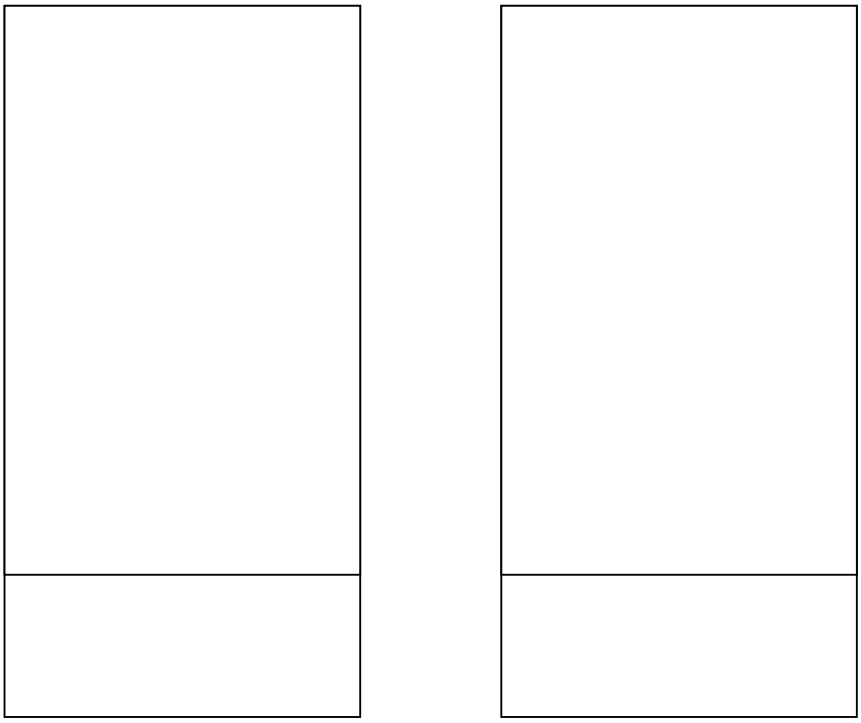
Experiment 4: Purity of Samples

In this experiment you will investigate the use of TLC as a measure of sample purity.

Place about 5 mg of the recrystallised aspirin sample in a semi-micro test tube and about 5 mg of salicylic acid in another semi-micro test tube. Add approximately 2 mL of methanol to each test tube and another 2 mL to the test tube containing your crude aspirin and stir until the solid has dissolved. Set up a TLC plate of your two aspirin samples, salicylic acid and an authentic sample of aspirin. Develop your TLC plate, as before, and examine it under UV light.

If you have previously completed E25, retrieve your sample of caffeine from your demonstrator. Set up a TLC plate and check the purity of your sample against the caffeine standard supplied.

Sketch your TLC plates below. Indicate which spots glowed under long wave-length UV light and which spots glowed under short wave-length UV light.



Comment on the purity of the samples.

Demonstrator's Initials	

Experiment 5: Water soluble aspirin

In this experiment you will investigate the chemistry behind how water soluble aspirin tablets, like Alka-Seltzer®, work.

Into a 100 mL conical flask, add 0.25 g of your recrystallised aspirin. Add 5 mL of water to the flask and swirl it. Does the aspirin dissolve?

Into another 100 mL conical flask, add 0.25 g of your recrystallised aspirin and 0.40 g of sodium hydrogencarbonate. Mix the solids together by swirling the flask. Add 5 mL of water to the flask and swirl it. Does the aspirin dissolve?

What reaction do you think is happening to make the aspirin water soluble? Write an equation to represent this reaction.

One tablet of Alka-Seltzer® contains aspirin (324 mg), sodium hydrogencarbonate (1.7 g) and citric acid (1.2 g). What do you think is the function of these two additives?

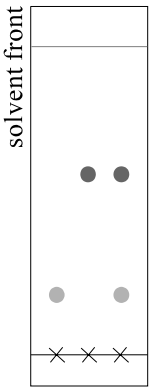
What do you predict would happen when water soluble aspirin enters your stomach? Hint: The pH of your stomach is about 1.

Demonstrator's Initials	

TLC TROUBLESHOOTING

Ideal Separation

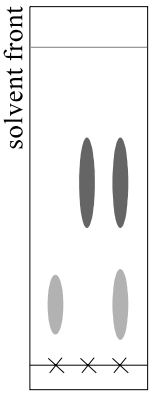
Sufficient sample has been applied in small, adequately separated spots.



Sample Streaking

Too much sample has been added, albeit in small spots causing the adsorbent to be 'overloaded'.

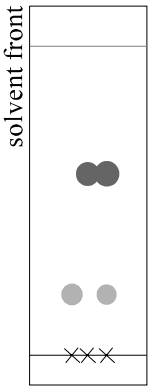
Add less sample.



Spots Overlapping Sideways

The chemist has placed the sample spots too close together on the origin.

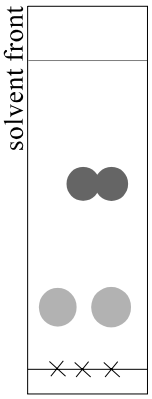
Use more space on the plate or use bigger (or several) plates.



Spots Too Big

The chemist left the spotting capillary in contact with the adsorbent for too long causing the sample to spread out.

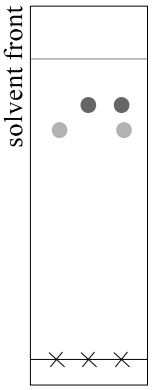
Add the sample solution with a fine capillary in short 'dabs'.



Spots Clustered Near Solvent Front

The elution solvent was too polar. The sample has migrated up the plate almost as fast as the solvent with little separation of the components.

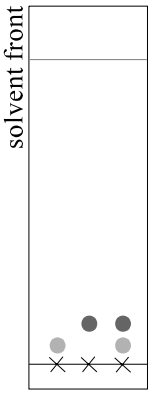
Use a less polar solvent.



Spots Clustered Near Origin

The elution solvent was insufficiently polar. The sample has not moved far up the plate with little separation because it is too strongly bound to the adsorbent.

Use a more polar solvent.



Spots Have Spread - no elution

The origin was below solvent level so no elution occurred. The spots have become smeared by diffusion.

Use less solvent in the jar or spot the samples higher up the plate.