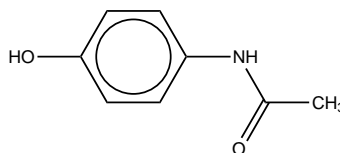


# Background information

## 1. Paracetamol is a common compound



### The structure of paracetamol

Paracetamol is a very widely used medicine. It is a mild painkiller and reduces the temperature of patients with fever. These actions are known respectively as analgesic and antipyretic.

There are currently more than 90 common products containing paracetamol which are available over the counter from British pharmacies. Many of them are sold as treatments for the relief of cold and influenza and they can be bought in a number of different formulations. Paracetamol is a relatively safe drug but toxic side effects have been observed with high doses greater than 10–15 g.

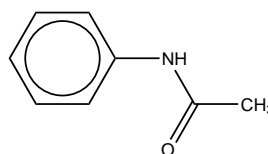
This toxicity is due to the chemical structure of the compound and the way our bodies break it down. It is metabolised to a reactive intermediate at high doses.

Pure paracetamol is a white crystalline solid which melts at 169–171 °C. Its solubility in cold water is 1.43 g/100 cm<sup>3</sup> but it is much more soluble in hot water (5 g/100 cm<sup>3</sup>) and in ethanol (14 g/100 cm<sup>3</sup>).

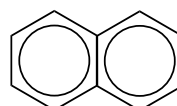
## 2. The history of paracetamol

At the University of Strassburg in the 1880s Professor Kussmaul, of the Department of Internal Medicine, asked two assistants to give naphthalene as a treatment for intestinal worms.

The medicine had little effect on worms, but one patient had a great reduction in fever temperature. It was found that this patient had, in fact, been given acetanilide instead of naphthalene due to a mistake at the pharmacy!



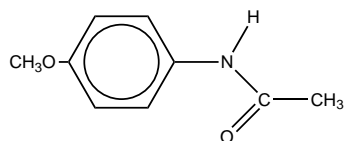
Acetanilide



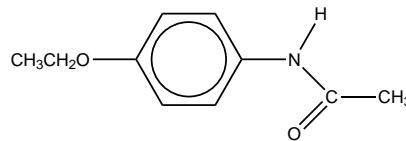
Naphthalene

The young assistants quickly published the discovery of this new antipyretic (fever-reducing drug). It was soon in production and remained in use for several years because it was so cheap to produce. However, it had a serious side effect involving the deactivation of some of the haemoglobin in red blood cells.

The publication of news about acetanilide immediately spurred a chemist at Bayer's dyeworks to make some derivatives:



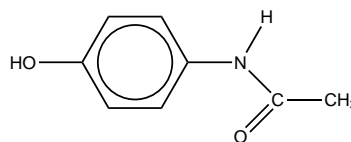
*N*-(4-Methoxyphenyl)ethanamide



*N*-(4-Ethoxyphenyl)ethanamide  
(Phenacetin)

These were both found to be antipyretic and *N*-(4-ethoxyphenyl)ethanamide was less toxic than acetanilide itself. It was promptly marketed as 'Phenacetin' and has remained in use ever since. However, restrictions have been placed on its use due to kidney damage in long-term users.

Many medicines were synthesised to try to improve on phenacetin and as early as 1893 Joseph von Mering made paracetamol.



*N*-(4-Hydroxyphenyl)ethanamide  
(Paracetamol)

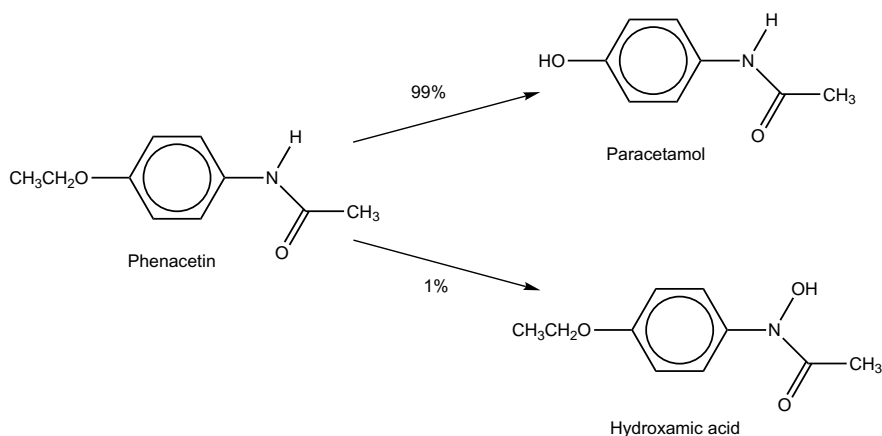
He found it to be an effective antipyretic and analgesic, but wrongly thought that it caused the same haemoglobin problem as acetanilide.

It was not until the 1940s that paracetamol was reinvestigated after it was found present in patients dosed with phenacetin. In 1953 paracetamol was marketed by Sterling-Winthrop Co., and promoted as preferable to aspirin since it was safe to take for children and people with ulcers. However, it causes liver damage from chronic use.

Paracetamol is rapidly formed in the guts of people who take phenacetin. It is the major metabolite (decomposition product) and it is likely that the antipyretic and analgesic effects of phenacetin were in fact due to paracetamol.

There are some suggestions that the toxic effects of phenacetin were due to a minor metabolite – the *N*-oxide.

Phenacetin is metabolised to two compounds. One involves the removal of the ethyl (CH<sub>3</sub>CH<sub>2</sub>-) substituent from oxygen. The second involves the replacement of the hydrogen atom on nitrogen by a hydroxyl (-OH) group. This type of compound is called a hydroxamic acid. Hydroxamic acids bind strongly to metal ions. This action may contribute to the toxicity.



### Methods of establishing the safety and efficacy of medicines

At the turn of the 20th century the discovery and testing of medicines was a largely haphazard process with the pioneers of high quality science such as Pasteur and Ehrlich being exceptions. As in the history of paracetamol, new compounds were given to patients almost immediately after synthesis or discovery.

In contrast, the development of modern medicines is a long and expensive process typically taking 10-12 years. Chemistry is used at all stages to develop the synthesis and determine purity. Potential patients need to know at least four things about a new medicine before they take it. Does it work? Is it safe? How much do I take? How often do I take it?

The answer to the first question is initially investigated in isolated enzyme or cellular systems before being trialed in animal models and ultimately in volunteer patients. Clinical trials are used in the later stages to see if a new medicine works in one set of patients compared to the effects of a placebo on another group.

The only way to determine safety and dosage regimes are to use animal models before human volunteers take the medicine.

#### Task

Consider the ethical considerations of testing new medicines on animals.

# Experimental and investigative section

## The extraction and purification of paracetamol from tablets

This can be used to find out which brand of paracetamol contains the greatest quantity of active ingredient.

### Safety

Wear eye protection.

**Propanone** – volatile, highly flammable, keep away from flames, do not inhale vapour.

**Paracetamol tablets** – do not ingest.

A full risk assessment should be carried out prior to starting the experiment.

- Warm two paracetamol tablets with propanone ( $20 \text{ cm}^3$ ) in a small conical flask by placing the flask in warm water.
- Once the tablets have broken up, the undissolved material (binding agents and filler) should be removed using a filter paper and funnel. Allow the propanone to evaporate, either overnight or on a warm water bath in a fume cupboard.
- The white solid is crude paracetamol. Keep a small amount of the solid to determine its melting point later. The material can be purified by recrystallisation from water.

This process relies on the fact that paracetamol is not very soluble in cold water ( $1.4 \text{ g}/100 \text{ cm}^3$ ) but very soluble in hot water ( $5 \text{ g}/100 \text{ cm}^3$ ). When the crude solid is heated in water it will dissolve and any insoluble impurities can be filtered off. The impurities which are soluble will also of course dissolve. When the hot solution is cooled down, it reaches the temperature at which paracetamol reaches its limit of solubility and therefore starts to crystallise out. However, the soluble impurities are only present to the level of a few percent and so never reach their limit of solubility and thus stay in solution.

- Heat the solid in about  $10 \text{ cm}^3$  of water to dissolve it, and filter off any insoluble material through a very small piece of cotton wool in a warm glass funnel. (Pour hot water through the funnel and cotton wool first.)
- Cool the filtrate, and filter off the crystals that form.
- Dry the pure paracetamol by either pressing with filter papers or gently warming in an oven. Take the melting point, and compare it with that of your crude sample and the quoted melting point of pure paracetamol.

### Question

Why might the melting points be different?

# Paracetamol formulations: presentation activity

Paracetamol can be taken in a number of ways and can be bought in many different formulations. Common ones are tablets (500 mg), fizzy dispersible tablets (500 mg), paediatric oral solutions (120 mg/5 cm<sup>3</sup>), oral suspensions (250 mg/cm<sup>3</sup>), and suppositories (125 mg). It is also sold in capsules as a mixture with other active ingredients such as codeine and caffeine. Work in small groups and discuss why there might be advantages in having a number of formulations. Comment on the doses available and suggest a target group for each one. Present your findings to the group, perhaps as a poster or as an audio-visual presentation eg Powerpoint presentation.

## Hints on presentations

In the presentation you could include the following:

- the conditions that paracetamol helps to relieve or cure, including technical terms such as analgesic, antipyretic and anti-inflammatory;
- the side effects of paracetamol, and the alternative treatments for people who are affected by them;
- the historical development of paracetamol, including the achievements of those responsible for the main developments;
- the chemistry involved in developing the medicine in a usable form; and
- the nature and importance of clinical trials.

You may find information in reference books, in libraries, in pharmacies and by contacting the ABPI (Association of the British Pharmaceutical Industry, 12 Whitehall, London, SW1A 2DY, <http://www.abpi.org.uk> Tel: 020 7930 3477) or pharmaceutical companies.

A recommended website is <http://www.pharmweb.net/pwmirror/pwy/paracetamol/pharmwebpic.html> (accessed August 2002).

## Making a poster

In making a poster the following hints may be useful:

- your poster should be clearly set out, the structure should be clear at a glance;
- people do not like reading a lot of text. Diagrams and flow charts are much easier to take in; text should be readable from at least 2 m;
- explanations should be separate from the main story, perhaps in distinctive boxes; and
- the level must be appropriate for the expected audience: you will need to think about what the audience is likely to know already.

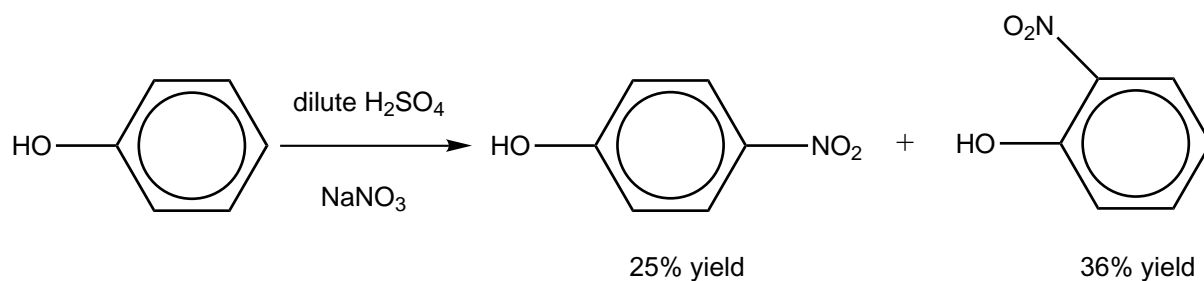
## Making an audio-visual presentation

In making an audio-visual presentation the following hints may be useful:

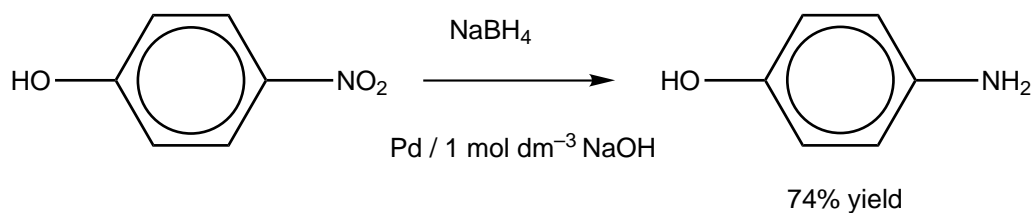
- before you start, make sure you have everything ready and you know how to switch on the OHP or operate the data projector and that it is focussed correctly;
- start the presentation with something designed to capture attention and to help your audience to know what to expect;
- do not read directly from notes: use notes if you need to, but always talk directly to your audience;
- people get bored if they have nothing to do but listen to you talking: make sure that there is always something to look at as well;
- make sure your visual aids are prepared well beforehand: they are a very effective way of getting information across to your audience;
- if you are drawing formulae on a white board or black board make sure that you know them by heart (draw them out beforehand): you should not have to keep looking at your notes to make sure that you have got something right;
- remember that you are always more familiar with your subject matter than your audience: give them time to take in what you are saying before going on to the next stage; and
- mannerisms are irritating, so try to stand still, look at your audience and do not wave your hands about, or keep scratching your nose or trip over the OHP lead!

# The preparation of paracetamol

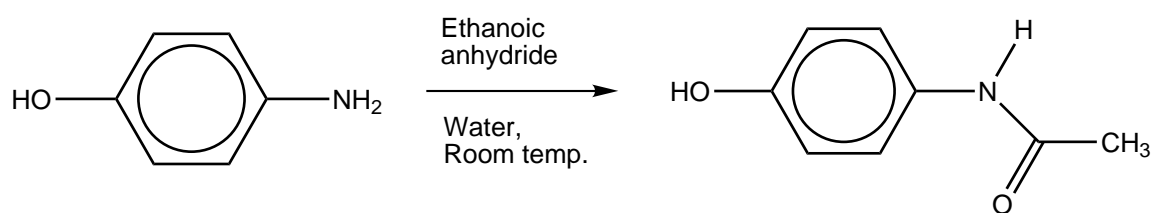
Paracetamol can be made in three steps from phenol



Step 1



Step 2



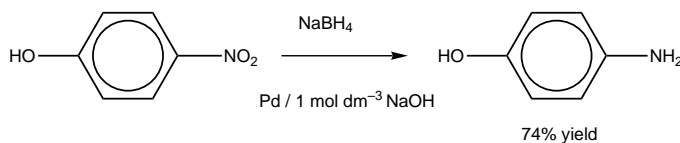
Step 3



8. Look up the melting points of the following pairs of compounds (be careful how you interpret the names).
- 3-methyl-2-nitrophenol
  - 3-methyl-4-nitrophenol
  - 5-methyl-2-nitrophenol
  - 3-methyl-4-nitrophenol
  - 5-fluoro-2-nitrophenol
  - 3-fluoro-4-nitrophenol

What do you notice about the melting points?

# Step 2 – the reduction of a nitro group to an amine



## Preparing 4-aminophenol

### Safety

Wear eye protection.

**Sodium tetrahydridoborate(III)** – harmful if swallowed, reacts with water to produce hydrogen; the reaction is more vigorous with acids – keep away from flames.

**4-nitrophenol** – irritant, will stain skin, wear gloves when handling.

**Sodium hydroxide solution** (1 mol dm<sup>-3</sup>) – corrosive – avoid contact with eyes.

**Palladium on charcoal** (5% or 10%) – Irritant. Do not breathe dust.

**Hydrochloric acid** (2 mol dm<sup>-3</sup>) – irritant.

**Sodium hydrogen carbonate** – reacts with acids to produce carbon dioxide. The reaction can be delayed and violent. Add cautiously with constant agitation and hold the flask over a dish to catch any spill-over.

**4-aminophenol** – harmful, possible risk of irreversible effects, very toxic to aquatic organisms.

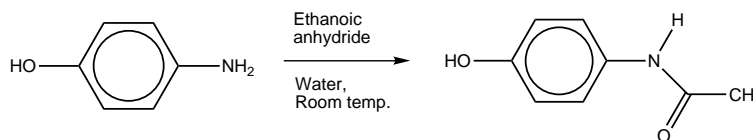
Note – This reaction needs careful temperature control.

1. Place 10 cm<sup>3</sup> (10 mmol) of 1 mol dm<sup>-3</sup> sodium hydroxide in a conical flask.
2. Add 0.56 g (14.7 mmol) of sodium tetrahydridoborate(III) (sodium borohydride), followed by 50 mg of palladium on charcoal (5% or 10%; Aldrich).
3. Cool in ice to ~13 °C.
4. Add 1.0 g (7.2 mmol) of 4-nitrophenol in very small portions (half a microspatula at a time) over 30 minutes. Make sure the temperature is kept between 13–17 °C during the addition.
5. After the addition is complete the mixture should be stirred for a further 15 min and acidified with 2 mol dm<sup>-3</sup> hydrochloric acid (about 17 cm<sup>3</sup>).
6. Filter the mixture to remove catalyst and adjust the filtrate to pH 7–8 by carefully adding solid sodium hydrogencarbonate a little at a time.
7. Filter off the precipitate and wash with a little cold water to give 4-aminophenol (0.58 g; 74%) after drying.

### Questions

1. Sodium tetrahydridoborate(III) (sodium borohydride), NaBH<sub>4</sub> is relatively stable in aqueous sodium hydroxide (NaOH) but not in acid. Why?
2. What is the role of the catalyst in the reduction of nitro groups? Can other methods be used for this reduction?
3. Why can the product be separated from unreacted starting material at pH 8?
4. Why is sodium hydrogencarbonate used to make the reaction mixture basic at the end of the reaction, rather than sodium carbonate or sodium hydroxide?
5. Why is the product soluble in a solution of a strong acid or in a solution of a strong base, but not in a solution of a weak base?

# Step 3 – the formation of an amide



**Preparing N-(4-hydroxyphenyl)ethanamide – Paracetamol**

## Safety

Wear eye protection.

**4-aminophenol** – irritant.

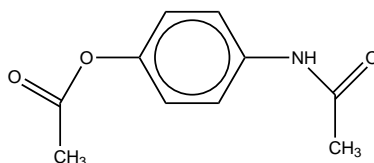
**Ethanoic anhydride** – corrosive, causes burns, flammable, the vapour will irritate the eyes and the respiratory system. Use in a fume cupboard.

**Paracetamol** – do not ingest.

1. Place 1.0 g of 4-aminophenol and 9 cm<sup>3</sup> of distilled water in a 50 cm<sup>3</sup> conical flask and stir briskly at room temperature, in order to suspend the solid in the water.
2. In a fume cupboard, add 1.1 cm<sup>3</sup> (1.17 g) of ethanoic anhydride to the stirred suspension and gently shake to mix. The solid will dissolve after about 30 seconds. Continue shaking and a precipitate will form after 2 minutes.
3. After 10 minutes the solid should be filtered off under suction, washed with a little cold water and dried (0.83g; 60%).
4. The product may be purified by crystallisation from distilled water. Dissolve the crude product in the minimum of distilled water at about 80 °C (you will probably require about 15 cm<sup>3</sup>).
5. Allow the clear solution to cool slowly to room temperature and collect the recrystallised product by suction filtration, washing with 5 cm<sup>3</sup> of ice-cold distilled water.
6. Dry the recrystallised product either between filter papers or by gently warming in an oven, and determine the yield.
7. Determine the melting point of the dry, recrystallised product. The melting point should be 169–171 °C.

## Questions

1. Why is the product insoluble in aqueous ethanoic acid, but the starting material is soluble?
2. If the reaction is done in dilute hydrochloric acid rather than water, the product is

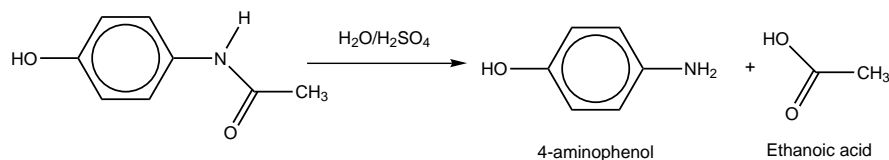


Explain why this is.

## The quantitative analysis of various formulations of paracetamol

The British Pharmacopoeia method for the analysis of paracetamol involves heating it under reflux with  $1 \text{ mol dm}^{-3}$  sulfuric acid. This is a straightforward, acid catalysed, hydrolysis of an amide to an amine and a carboxylic acid. The 4-aminophenol which is formed is then titrated with an oxidising agent, ammonium cerium(IV) sulfate using ferroin as the indicator.

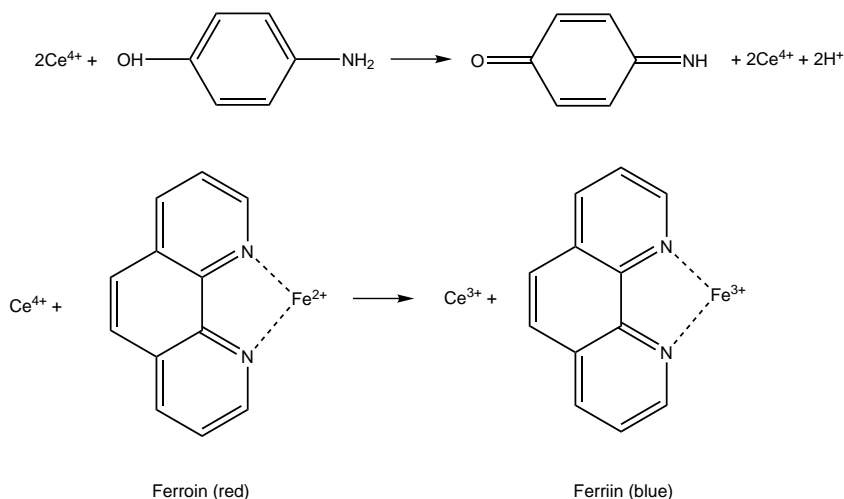
The first reaction is as follows:



The titration step is much more interesting. 4-Aminophenol can easily be oxidised as follows:



The role of the ammonium cerium(IV) sulfate is to oxidise the 4-aminophenol to the iminoquinone. Only after all the 4-aminophenol has been oxidised will the cerium (IV) reagent oxidise the ferroin indicator from  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  (ferriin).



During the titration the solution should be red, and the yellow end point is the transition from red to pale blue.

It is easy to work out that, since 1 mole of  $\text{Ce}^{4+}$  is equivalent to 0.5 mole of paracetamol, the conversion factor given in the method is correct.

## Procedure as outlined in the British Pharmacopoeia 1988

### Safety

Wear eye protection.

**Paracetamol formulations** – do not ingest.

**Sulfuric acid** ( $1 \text{ mol dm}^{-3}$ ) – corrosive, especially when hot.

**Hydrochloric acid** ( $2 \text{ mol dm}^{-3}$ ) – irritant.

**Ferroin solution** – hazards unknown. May cause skin irritation.

**Ammonium cerium(IV) sulfate** – respiratory tract irritant, strong oxidising agent, keep away from flammable material.

1. Dissolve 0.3 g of a mixture containing paracetamol in a mixture of water ( $10 \text{ cm}^3$ ) and  $1 \text{ mol dm}^{-3}$  sulfuric acid ( $30 \text{ cm}^3$ ).
2. Boil under reflux for 1 hour, cool and dilute with water ( $100 \text{ cm}^3$ ).
3. To  $20 \text{ cm}^3$  of the resulting solution add cold water ( $40 \text{ cm}^3$ ,  $2 \text{ mol dm}^{-3}$  hydrochloric acid ( $15 \text{ cm}^3$ ) and ferroin solution ( $0.1 \text{ cm}^3$ , 0.1 wt% or  $0.025 \text{ mol dm}^{-3}$ ).
4. Titrate with  $0.1 \text{ mol dm}^{-3}$  ammonium cerium(IV) sulfate (VS – volumetric standard) until a yellow colour is produced.
5. Repeat the operation without the test material being present. The difference between the titration figures represents the amount of ammonium cerium(IV) sulfate required. Each  $\text{cm}^3$  of  $0.1 \text{ mol dm}^{-3}$  ammonium cerium(IV) sulfate is equivalent to 0.007560 g of paracetamol.

This method can be used to analyse the quantity of paracetamol present in many medicines that contain the drug.

### Question

How could you prove that the first step in the quantitative analysis of paracetamol involves hydrolysis to 4-aminophenol?

## Using thin-layer chromatography to investigate the reactions

You have probably used a simple chromatography experiment as part of your earlier studies to separate the dyes in a coloured ink. The same technique can be used to separate substances which are not dyes but in such experiments the chromatogram must be developed to show up the various different substances that have been separated.

Chromatography techniques are used a great deal in industry because they can be controlled very precisely and use very small amounts of substance. In this activity you investigate the purity and identity of your laboratory prepared samples of nitrophenol or paracetamol using thin-layer chromatography (tlc). In this activity all the substances are white or pale yellow so you will need to develop the plate before you can see what has happened.

Thin-layer chromatography is a powerful tool for determining if two compounds are identical. A spot of the compound being investigated is placed on a chromatography plate, and a spot of a pure manufactured sample of the same substance is placed next to it. The plate is then allowed to stand in a suitable solvent, which travels up the plate. If the compound to be identified leaves exactly the same pattern on the chromatography plate as the known pure compound it is reasonable to conclude that they are the same. However, if extra spots are observed as well as the characteristic pattern of the known compound, then impurities are likely to be present in the sample.

In the experiment both crude samples of 2-nitrophenol and 4-nitrophenol are compared with known samples.

### Safety

Wear eye protection.

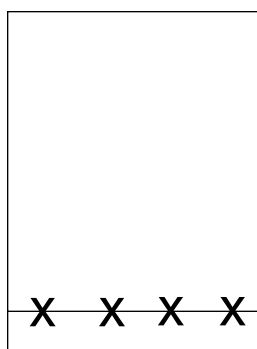
**Ethyl ethanoate** – volatile, highly flammable, keep away from flames, irritant, do not inhale vapour.

**Cyclohexane** – volatile, highly flammable, keep away from flames, harmful, do not inhale vapour.

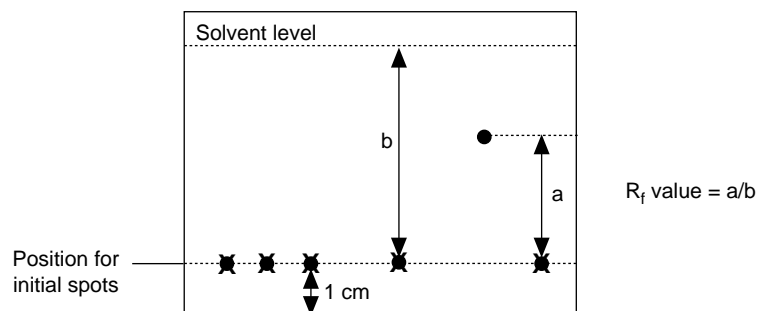
**Iodine** – harmful by inhalation or skin contact.

**Short wave UV** – may cause skin cancer and eye damage. Do not observe directly. The viewer should be screened from direct radiation.

1. Make sure that you do not touch the surface of the tlc plate with your fingers during this activity. Handle the plate only by the edges and use tweezers if possible.
2. Take a tlc plate and using a pencil (not a ball-point or felt tip pen) lightly draw a line across the plate about 1 cm from the bottom. Mark four equally spaced points on this line.
3. Place small amounts (about 1/3 of a spatula measure) of your crude 2-nitrophenol, your crude 4-nitrophenol and the commercial samples of these in four separate test-tubes. Label the test-tubes so that you know which is which.



4. Add 1 cm<sup>3</sup> of ethyl ethanoate to the test-tubes to dissolve the samples. If possible do this in a fume cupboard.
5. Use clean capillary tubes to spot each of your four samples onto the tlc plate. Allow the spots to dry and then repeat. The spots should be about 1–2 mm in diameter.
6. After all the spots are dry, place the tlc plate in the chromatography tank making sure that the original pencil line is above the level of the developing solvent – ethyl ethanoate:cyclohexane 1:4. Put a lid on the tank and allow to stand in a fume cupboard until the solvent front has risen to within a few millimetres of the top of the plate.



7. Remove the plate from the tank and quickly mark the position of the solvent front. Allow the plate to dry.
8. Observe the plate under a short wavelength UV lamp and lightly mark with a pencil any spots observed.
9. Carefully place the plate in a jar or beaker containing a few iodine crystals. Put a cover on the jar and wait for the spots to appear. Do this in a fume cupboard if possible.

#### Results

- Draw a diagram to show which spots appeared under UV light and which appear with iodine.
- Determine the R<sub>f</sub> value of the samples using the expression

$$R_f = \text{distance moved by sample} / \text{distance moved by solvent}$$

#### Questions

1. Write a short paragraph explaining why some substances move further up the TLC plate than others and how the results are made visible.
2. What conclusions can you draw about the nature of the four samples tested?

#### Other experiments

The conversion of 4-aminophenol into paracetamol can be followed using ethyl ethanoate:cyclohexane 2:1 as the eluant.

This system can also be used to monitor the hydrolysis step in the quantitative analysis.

To test aqueous mixtures, take a small sample (less than 1 cm<sup>3</sup>) and place in a test-tube. Adjust the solution to pH 8 by adding small amounts of sodium hydrogen carbonate. Add ethyl ethanoate (1 cm<sup>3</sup>) and shake. Allow the layers to separate and use a capillary tube to sample the upper ethyl ethanoate layer for TLC.

Control of the pH of the aqueous sample is important to ensure the organic compound is not ionised otherwise it will not extract effectively or run on a TLC plate. Use known samples of 4-aminophenol and paracetamol as reference samples on the plate.

This could form a separate investigation to semi-quantitatively look at aqueous pH versus how much can be extracted with ethyl ethanoate. Look at the spot size after running a TLC. Investigate paracetamol, 4-aminophenol and 4-nitrophenol.

# Appendix

## Column chromatography – the separation of 2- and 4-nitrophenols

### Safety

Wear eye protection.

Use a column with a sintered glass disc.

**Ethyl ethanoate** – volatile, highly flammable, keep away from flames, irritant, do not inhale vapour.

**Cyclohexane** – volatile, highly flammable, keep away from flames, harmful, do not inhale vapour.

**Silica** – its dust is a respiratory tract irritant.

**Petroleum ether** 60–80 °C – harmful, highly flammable

(It is possible to use solid phase extraction cartridges (10 g silica) and reduce the scale)

A full risk assessment should be carried out prior to starting the experiment.

Add sand to the disc to a depth of 5–10 mm, and add the solvent mixture to fill the column to about one-third. The solvent is ethyl ethanoate:cyclohexane 1:4 (petroleum ether 60–80 °C can be used instead of cyclohexane)

Weigh out silica (50 g) (in a fume cupboard and wearing gloves) and add enough solvent to give a mobile (runny) slurry. Pour this gently into the column with the tap open. Run the solvent down to the top of the silica while tapping the column a few times to pack the particles closely in it. Gently add another 5–10 mm layer of sand to the top of the material in the column.

Dissolve a mixture of the nitrophenols (0.25 g of each) in ethyl ethanoate (0.25 cm<sup>3</sup>) and dilute with cyclohexane (2 cm<sup>3</sup>). Carefully apply the solution to the sand on top of the column. Run the mixture onto the silica by opening the tap slightly and gently adding solvent. Once the phenols are on the silica and off the sand, fill the column with solvent, adding it very slowly at first so as not to disturb the top layer.

Start running the column by opening the tap, using a modest drip rate, and collect fractions in test-tubes. Keep topping up the solvent head at this point.

Check each fraction by tlc using the same solvent mixture, and combine the fractions which contain the same component of the mixture. Evaporate off the solvent, by leaving the solution in a small open vessel in a fume cupboard.