Student's document

PAIN RELIEF: Parallel synthesis of paracetamol analogues

Pharmaceutical companies synthesise and test tens of thousands of compounds in the search for one that shows significant therapeutic biological activity. Carrying out these syntheses efficiently and effectively is crucial. Combinatorial chemistry is used - in particular, a form of combinatorial chemistry called parallel synthesis. This is often done using robotic systems, but a few manual parallel syntheses are also carried out. Only small quantities are needed. For example, using plastic well plates, ten tests for biological activity can be carried out with only 1 mg of compound.

Your brief

Paracetamol is one of a number of over-the-counter (OTC) pain relievers. In this activity, you will work in a small group. Each of you will prepare a sample of paracetamol. Then, between you, members of the group will investigate the microscale preparation of paracetamol and the formation of a range of organic compounds structurally related to paracetamol. You will examine the reaction mixtures using thin layer chromatography.

From your results, the team needs to provide advice to the research and development (R&D) management team about which reactions appear to give products and, therefore, might be worked up to provide samples that can be tested for biological activity. This advice must also come with a costing exercise, saying how much it would cost to make these compounds.

Your investigation

Work in a group of four. Your group will be provided with three **sheets**:

- *Preparation of paracetamol*
- *Microscale investigation of paracetamol synthesis*
- *Parallel synthesis of compounds structurally similar to paracetamol*

Planning

Each member of the group should make a sample of paracetamol on a semi-micro scale (*Preparation of paracetamo*l). Compare your results - percentage yield and melting point of recrystallised paracetamol.

Next you need to work as a group to carry out the microscale synthesis of paracetamol and the parallel synthesis of some organic compounds that are structurally similar to paracetamol.

Each member of the group should read the **sheet** *Microscale investigation of paracetamol synthesis*. Make sure you are all clear about the instructions for the microscale preparation and the thin layer chromatography investigation of the reaction mixture.

Now read *Parallel synthesis of compounds structurally similar to paracetamol*. Check with your teacher what chemicals are available for you to use.

Plan what you are going to do and share out the work between group members. The group will now carry out the type of manual parallel synthesis usually done

by a single chemist. In parallel synthesis as little as 5 mg of a compound is made. High yield is not important. The desired compound is separated and purified by chromatography.

Your group will share the task of making an array of compounds (sometimes called a library) that are structurally related to paracetamol. How many there are in the array depends on the chemicals available and your ability to manage time and workload.

Practical work
Once you have decided what each member of the group will be doing, set up your work area and collect the equipment and materials you need.

You must be sure a risk assessment has been carried out before starting work.

Information
- You will need to find the prices of chemicals from the catalogues of chemical suppliers. Examples include:
  http://uk.vwr.com/app/Home
  http://www.sigmaaldrich.com/Area_of_Interest/Europe_Home/UK.html

Your findings
Write a brief report that includes the group's findings. While writing your report, imagine you are writing it to the R&D management team of a pharmaceutical company. You should say which reactions appear to give products and, therefore, might be worked up to provide samples that can be tested for biological activity. You must also include calculations showing an estimation of how much it would cost to make these compounds.

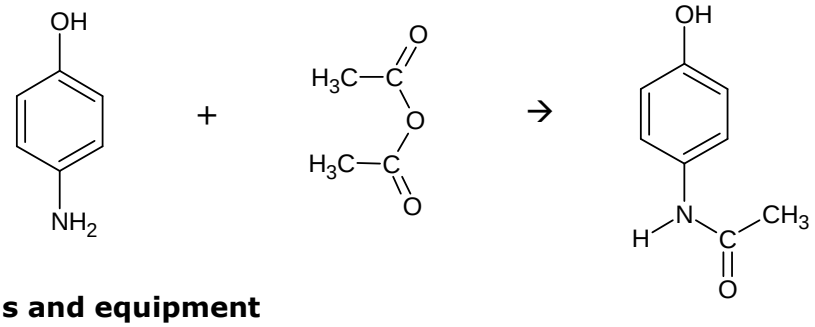
Attach to your report:
- the results of the semi-micro scale preparation of paracetamol
- the summary table showing the outcomes from the parallel syntheses of compounds structurally related to paracetamol.

Student self assessment
This activity was about using scientific knowledge and understanding to solve problems, working in teams to solve problems, managing time and workload, resources and budgets. For each case, write about 50-100 words explaining:
- what scientific skills and knowledge you used and how effectively you used them
- how you worked as a team and give examples of how you supported others in your group or were supported by them
- how you managed your time and workload
- how you managed resources and budgets.

Student sheet: Preparation of paracetamol

Paracetamol is a commonly used analgesic. Aspirin and ibuprofen are the two other most common analgesics. The chemical name for paracetamol is N-(4-hydroxyphenyl)ethanamide.

It may be prepared in the laboratory by reacting 4-aminophenol with ethanoic anhydride.



Materials and equipment

- 4-aminophenol
- ethanoic anhydride
- ethyl ethanoate
- balance weighing to the nearest 0.01 g
- 50 cm^3 conical flask
- Buchner flask, funnel and filter paper
- clamp and stand (to hold the Buchner flask)
- vacuum pump (water pump)
- apparatus for determining melting point
- distilled water wash-bottle
- Bunsen burner, tripod and gauze
- boiling tube
- 400 cm^3 beaker (for a water bath)
- equipment for thin layer chromatography (see *Microscale investigation of paracetamol synthesis*)
- access to a fume cupboard

Procedure

Eye protection must be worn. A risk assessment must have been made before starting this practical work.

1. Weigh 1.0 g of 4-aminophenol into a 50 cm^3 conical flask. Add 9 cm^3 of distilled water and shake the mixture briskly to suspend the solid in water.

2. In a fume cupboard, add 1.1 cm^3 (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, filter off the solid under suction filtration, wash with a little cold water and dry.

Note: Use a Buchner flask and funnel connected to a vacuum pump (water pump). Clamp the flask to keep it stable. Put a filter paper in the funnel, attach the flask to the pump and pour through about 10 cm^3 water as a practice run

and to wet the filter paper. Remove the vacuum tube, pour out the water and then do the real filtration. If you do not manage to transfer all of the solid from the reaction mixture into the funnel at the first attempt, just pour the filtrate back into the conical flask and repeat the process.

Remove a small sample (A), dissolve in minimum quantity of ethyl ethanoate and keep in a labelled sample tube.

4. To recrystallise the sample, put it into a small conical flask. Dissolve the crude product in the minimum volume (probably about 15 cm^3) of distilled water. Heat it in a hot water bath at 80 °C until the solid dissolves. Add a few extra drops of hot water, if necessary, to dissolve the solid.

5. Cool the solution under a tap or preferably in ice. Collect the recrystallised product by suction filtration, washing with 5 cm^3 of ice-cold distilled water.

6. Dry the recrystallised product between filter papers and then in an oven (about 105 °C). Determine the yield. Calculate the percentage yield.

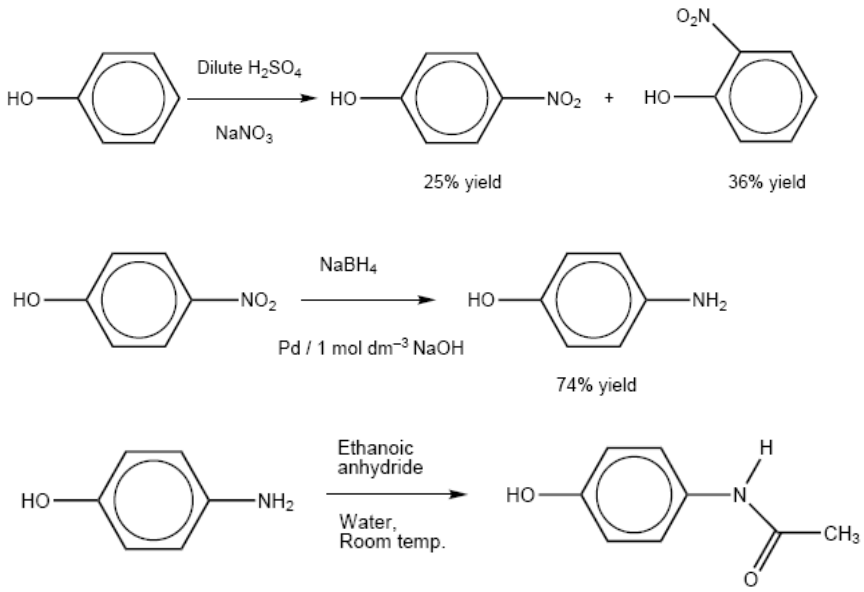
Remove a small sample (B), dissolve in minimum quantity of ethyl ethanoate and keep in a labelled sample tube.

7. Find the melting point of the dry, recrystallised product. The melting point of pure paracetamol is 169-171 °C.

8. Now run a thin layer chromatogram using the method in *Microscale investigation of paracetamol synthesis*. Run the crude paracetamol (**A**) and recrystallised paracetamol (**B**) against (a) 4-aminophenol, (b) pure paracetamol and (c) a mixture of 4-aminophenol and paracetamol (all dissolved in ethyl ethanoate).

Results

- Calculate the percentage yield of recrystallised paracetamol.
- Record the melting point of the recrystallised paracetamol.
- Sketch a diagram of the chromatogram obtained (or put the chromatogram in a transparent plastic bag and fix it in your notebook).
- Compare your results with other members of your group and explain any differences.
- Look at this scheme for making paracetamol from phenol:



Using the data shown together with the percentage yield you estimated for the conversion of 4-aminophenol to paracetamol, calculate the yield of paracetamol that might be obtained from 50 g of phenol.

Student sheet: Microscale investigation of paracetamol synthesis

Preparation

Materials and equipment
- 4-aminophenol
- ethanoic anhydride
- anhydrous magnesium sulfate
- ethyl ethanoate
- distilled water
- 2 x small sample tubes
- 3 x dropping pipettes
- balance weighing to the nearest 0.001 g
- access to a fume cupboard

Procedure
Eye protection must be worn. A risk assessment must have been made before starting this practical work.

1. Using a weighing boat measure out 0.1 g of 4-aminophenol into a small sample tube. Add 1 cm^3 of distilled water. Shake briskly at room temperature in order to suspend the solid in water.

2. In a fume cupboard, add 1 drop (about 0.12 g) ethanoic anhydride to the suspension and continue to shake the mixture gently for 2 minutes. (You could check the weight of 1 drop from your pipette before doing this).

3. Add 1 cm^3 ethyl ethanoate and shake gently for 10-15 seconds. Leave to settle. Two layers will form. The product is in the ethyl ethanoate upper layer (the lower layer is mainly water).

4. Using a clean dropping pipette, carefully transfer the upper layer to another sample tube containing a small amount of anhydrous magnesium sulfate. This will remove any traces of water.

5. Now use thin layer chromatography (see method below) and run this solution against (a) 4-aminophenol in ethyl ethanoate, (b) pure paracetamol in ethyl ethanoate, and (c) a mixture of 4-aminophenol and paracetamol in ethyl ethanoate.
In each case, make these solutions by dissolving half a small spatula of material (equal to about 1-2 grains of rice) in 1 cm^3 ethyl ethanoate.

Thin layer chromatography (TLC)

Materials and equipment

- solvent mixture – 2 parts by volume ethyl ethanoate and 1 part by volume cyclohexane
- iodine
- TLC plates (coated in silica gel and containing a fluorescing agent)
- screw-top jar large enough to take a 10 cm x 5 cm TLC plate
- finely drawn glass tubing (for spotting the plates)
- UV lamp

Procedure

Eye protection and gloves must be worn. The ultraviolet radiation may cause skin cancer and eye damage - do not observe directly. A risk assessment must have been made before starting this practical work.

1. Pour the solvent mixture into a screw-top jar to a depth of about 0.5 cm. Put the lid on and leave so that the atmosphere becomes saturated with the solvent vapour. This process could be accelerated by laying a piece of filter paper soaked in the solvent mixture on the inner wall of the screw-top jar. The atmosphere should be saturated with solvent vapour in 30 minutes.

2. Lay a 10 cm x 5 cm TLC plate on a piece on clean paper. Make sure you handle the plate by the edges only; do not touch the surface. Draw a pencil line across the plate about 1 cm from the bottom. On three small pencil crosses, spot first the product, then a mixture of your product and starting material and finally 4-aminophenol. Allow the spots to dry – they should be 1-2 mm in diameter. This process could be accelerated by using a hairdryer.

3. When the spots are dry, place the TLC plate in the screw-top jar. Do this quickly, but carefully. Try not to disturb the liquid. Immediately replace the lid on the jar.

Note: The original pencil line must be above the level of the solvent.

4. Run the chromatogram until the solvent front is within about 1 cm of the top of the plate. Remove the plate and mark the position of the solvent front.

5. Allow the plate to dry. Observe under UV light and mark any spots seen. Calculate the R_f values of the spots observed.

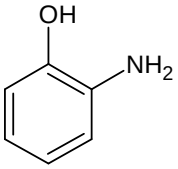
6. Finally, if time, place the chromatogram in a covered beaker containing a few crystals of iodine and wait for spots to appear.

Student sheet:
Parallel synthesis of compounds structurally similar to paracetamol

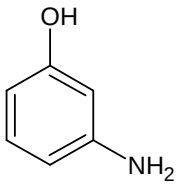
The microscale method for exploring the reaction between 4-aminophenol and ethanoic anhydride may be adapted to investigate the reaction of various compounds that are structurally similar to 4-aminophenol with various acid anhydrides.

You will need to check with your teacher what chemicals are available. However, here are some that may be:

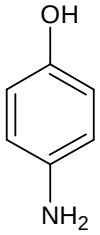
Possible aminophenols



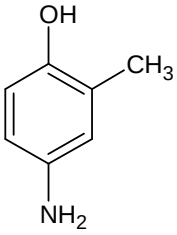
2-aminophenol



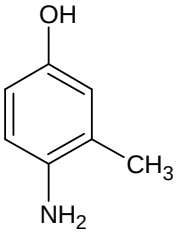
3- aminophenol



4- aminophenol

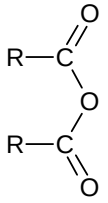


4-amino-2-methylphenol



4-amino-3-methylphenol

Possible acid anhydrides



R = CH_3 ethanoic anhydride (acetic anhydride)

R = C_2H_5 propanoic anhydride (propionic anhydride)

You can plan and record your results in a table similar to the one on the next page. Copy it and fill in the aminophenols used by members of your group. Indicated clearly which one(s) you investigated.

In each box, record:

- The initials of the group member who investigated
- The name and chemical formula of the aminophenol used
- The chemical structure of the compound you expected to be formed
- Evidence for new compound formation (or not) from the TLC studies
- The cost of making 1 g of the compound for further study

  Note: You will not have measured yields (in fact, the idea of parallel synthesis is simply to get evidence of product formation; optimising reaction conditions comes later). However, it is extremely unlikely that 100% yields would be obtained. Therefore, give costs based on 25%, 50%, 75%, and 100% yields.

- A prediction about the drug potential of the product.

  Note: Use the *OSIRIS Property Explorer* http://www.organic-chemistry.org/prog/peo/ to estimate a 'Drug-Likeness Prediction' and 'Overall Drug-Likeness Score'. You need to have Java installed to use the molecular modelling programme.

You may need more or less boxes depending on what reactions the group was able to study.

initials	aminophenol used	ethanoic anhydride	propanoic anhydride