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Teachers' Notes
Safety note to teachers and lecturers

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Additional points to note:

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Extraction of Natural Perfume Ingredients

Activity 1  Limonene from citrus fruits

The outer, coloured rind of citrus fruits contain oil glands, which you may just be able to see in the cross-section of the rind from a freshly cut fruit. Each oil gland contains the essential citrus oil and the cells that produce it. Limonene is a major component of citrus oils and makes some contribution to their odours. If you damage the rind the glands break open and release their contents.

D-limonene  L-limonene

You can demonstrate that the rind contains these oils by making the glands rupture near a Bunsen flame. Cut a section of rind forming an arc. Use a darkened room for the next step. Hold the rind at a distance of about 10 cm from a colourless Bunsen flame. With the outer layer facing the flame, bend the rind so that the outer layer becomes more convex. You should see small jets of yellow flame appear as the oil glands rupture and squirt the flammable oils into the flame.

In this series of experiments you will extract the citrus oil (containing limonene) from orange rind using the three techniques: expression, steam distillation and solvent extraction. These are the main methods used in the fragrance industry for extracting essential oils from natural materials. You can extend the work by extracting and investigating the citrus oils produced from other fruit, e.g. lemons, grapefruit and limes.

Part A: Expression

Requirements

- eye protection
- 2 or 3 oranges
- nutmeg grater
- garlic press
- spatula
- 2 x 250 cm³ beakers
- 2 x 250 cm³ conical flasks
- 1 x 100 cm³ conical flask
- filter funnel
- distilled water wash bottle
- centrifuge and centrifuge tubes
- 100 cm³ separating funnel
- 10 cm³ of petroleum ether (b.p. 60-80 °C)
- anhydrous sodium sulphate

Safety

Make sure you grate the peel and not your fingers.

Make sure the centrifuge tubes are balanced and the lid is secure while the centrifuge is spinning. If there is not an interlock to prevent the lid opening when power is on, do not use it.
Petroleum ether is highly flammable and harmful, there should be no naked flames in the laboratory. Avoid inhaling the petroleum ether vapour.

Work in a fume cupboard, at least during heating.

**What you do**

1. Weigh a clean, dry 250 cm$^3$ beaker.
2. Working over the 250 cm$^3$ beaker, use the grater to remove only the outer, coloured rind from the oranges.
3. Scrape the materials from the grater into the beaker with a spatula.
4. Re-weigh the beaker to determine the mass of grated rind you are starting with.
5. Gather some of the gratings into the garlic press and squeeze into the conical flask, fitted with a filter funnel.
6. Empty the squeezed pulp from the press into the flask.
7. Repeat the process until all of the grated rind has been pressed.
8. Use a total of about 20 cm$^3$ of distilled water to wash any residue from the grater and the press into the flask.
   - The flask now contains a mixture of essential oil, water and solid debris from the rind. In the next stage you will isolate the oil by removing the debris and water.
9. Use a centrifuge to separate the solid material. Make sure that the tubes you use are balanced by mass. Centrifuge for a longer period of time if the initial separation is unsatisfactory.
10. Carefully transfer the liquid to the separating funnel, taking care not to disturb the solid pellet at the bottom of each centrifuge tube.
11. Once you have allowed the liquid to settle you should see two layers. The uppermost is an emulsion of citrus oil and water. Add 10 cm$^3$ of petroleum ether to dissolve the oil, shake the mixture and allow it to separate before discarding the lower layer, which is mostly water.
12. Run off the remaining layer (citrus oil dissolved in petroleum ether) into a separate, dry conical flask and add enough anhydrous sodium sulphate to absorb any water, leaving the solution clear.
13. Transfer the solution into a clean, dry 100 cm$^3$ conical flask, leaving the sodium sulphate behind.
14. Working in a fume cupboard with no naked flames, evaporate the petroleum ether by placing the conical flask in a beaker of boiling water. (Heat the water using an electric kettle.)
15. Weigh a stoppered, clean, dry sample tube.
16. Transfer the oil into the tube, stopper it and weigh to determine the mass of oil you have extracted. Keep the oil in a cool, dark place, ready for analysis (see Part D).
Extraction of Natural Perfume Ingredients

Activity 1 Limonene from citrus fruits

Part B: Steam distillation

Requirements

See requirements for Part A, but include, in addition:

- steam generator
- tripod and gauze
- 2 x Bunsen burners
- 2 x stands and clamps
- 2 x 100 cm³ conical flasks
- 1 x 250 cm³ beaker
- 250 cm³ round-bottomed (or pear shaped) flask
- Leibig condenser and rubber tubing
- 100 cm³ measuring cylinder

Safety

Make sure that the water in steam generator does not evaporate away completely.

Make sure there is a vent tube in the generator and that there are no kinks in the tubing carrying the steam to the round-bottomed flask.

Petroleum ether is highly flammable and harmful, there should be no naked flame in the laboratory. Avoid inhaling the petroleum ether vapour by using a fume cupboard at least when it is heated.

What you do

1. Carry out steps 1 to 8 in Part A, but use a 250 cm³ pear-shaped or round bottom flask to collect the extract from the rind and the squeezed, pulped rind itself.

2. Add a further 100 cm³ of distilled water to the flask and set up the steam distillation apparatus as shown in the figure below.
3. Heat up the water in the steam generator to boiling, using a full Bunsen flame, then reduce the flame until it is sufficient to produce a steady supply of steam to the contents of the round-bottomed flask. At this point use a second Bunsen burner to gently heat the contents of the flask.

4. Collect at least 50 cm$^3$ of distillate. At this stage the citrus oil should form an oily emulsion on top of the aqueous layer in the receiver.

5. Continue the extraction following steps 11 to 16 in Part A.

You can obtain good yields without a separate steam generator.
Extraction of Natural Perfume Ingredients

Activity 1 Limonene from citrus fruits

Part C: Solvent extraction

In Part A and Part B you used a solvent to help you handle small quantities of oil you have already extracted. In this experiment you will use the same solvent to help perform the initial extraction from the grated rind.

Requirements

See requirements for Part A, but include, in addition:

1 x 250 cm$^3$ beaker
1 x 500 cm$^3$ beaker
1 x 250 cm$^3$ conical flask, fitted with a ground glass or plastic stopper
30 cm$^3$ of petroleum ether (b.p. 60-80 °C)
Access to a fume cupboard

Safety

Petroleum ether is highly flammable and harmful, there should be no naked flame in the laboratory. Avoid inhaling the petroleum ether vapour by using a fume cupboard at least for the evaporation stage.

What you do

1. Carry out steps 1 to 7 in Part A.
2. Carry out step 8 (Part A), but use a 250 cm$^3$ flask as the container.
3. Add 10 cm$^3$ of petroleum ether solvent to the flask, stopper and shake it vigorously for one minute. Remove the stopper from time to time to release the pressure created by the solvent evaporating.
4. Remove the stopper and allow the solid material to settle.
5. Without disturbing the solid, pour the liquid (both layers) through a filter funnel and into a separating funnel. You may find it necessary to place a tea strainer in the filter funnel to prevent solid material entering the separating funnel.
6. Allow the liquid in the separating funnel to form two separate layers.
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7. Run off the lower, aqueous layer back into the conical flask containing the solid residue from the orange rind. If you have used a tea strainer return the solid material from it into this flask as well.

8. Run the top layer, containing the oil dissolved in petroleum ether, into a clean, dry 250 cm³ beaker.

9. Repeat steps 3 to 8 twice using two separate 10 cm³ portions of petroleum ether. Combine all three extracts containing the solvent and the oil.

10. Add a spatula measure of anhydrous sodium sulphate and swirl the contents. Continue to add and swirl until all of the water has been absorbed and the extract appears clear.

11. Pour the oil extract into a clean, dry 250 cm³ beaker and leave in a fume cupboard and allow the solvent to evaporate - extinguish all naked flames. You may encourage evaporation by careful use of from an electric kettle. Avoid prolonged heating as this will evaporate the essential oil you are trying to extract.

12. Weigh a stoppered, clean, dry sample tube.

13. Once the solvent has evaporated completely transfer the remaining oil into the tube, stopper it and weigh to determine the mass of oil you have extracted. Keep the oil in a cool, dark place, ready for analysis (see Part D).
Comparison of the three different preparations on the citrus oil

Now you have obtained the essential citrus oil from orange rind you can evaluate the different extraction methods and compare the characteristics of the products you have obtained. These experiments are described in Part D, Part E, Part F and Part G.

Activity 1 Limonene from citrus fruits

Part D: Determination of yield

For each sample you have use the mass of product and the initial mass of the rind to calculate the percentage yield.

Which method of extraction do you think is most effective?

On an industrial scale, what factors, other than percentage yield, would a citrus oil producer need to consider before deciding which extraction method to use?
Activity 1  Limonene from citrus fruits

Part E: Description of fragrance characteristics

Requirements
5 mm wide strips of filter paper
Samples of the citrus oil extracts

What you do
You will need to approach this in an organised fashion, labelling each sample dip with its source and the time elapsed since dipping.

For an impression of top notes
For each sample:
1. Take a strip of filter paper and dip it in the sample so that the bottom 1 cm is wet with the oil (dip 1).
2. Smell straight away and describe the odour using the terms given in figure on http://www.schoolscience.co.uk/content/5/chemistry/smells/smellapa.html (the Lignes de Force diagram).
3. Smell the dip again after 15 minutes and describe any changes.
4. Smell the dip after a total of 30 minutes and compare with the smell of a fresh dip (dip 2). Describe the odour of the fresh dip. This is your impression of the top notes.

For an impression of body notes
1. Your description of the odour of dip 1 at 30 minutes is your provisional assessment of the body notes.
2. Leave dip 1 for a further 30 minutes then smell to make a final evaluation of the body notes. Compare with dip 2 for any odour differences.

For an impression of dryout notes
1. Continue to smell dip 1 at 30 minute intervals until the odour remains unchanged.
2. At this point make an assessment of the dryout notes.

(This is adapted from 'The Chemistry of Essential Oils' David G Williams, Micelle Press).
Activity 1 Limonene from citrus fruits

Part F: Determination of boiling point

Requirements
Samples of the citrus oil extracts
Thermometer (0 - 250°C)
Teat pipette
Melting point tube about 10 cm long and sealed at one end
5 mm diameter tube a little shorter than the melting point tube and sealed at one end
100 cm³ beaker
75 cm³ of medicinal paraffin
Electric hotplate

Safety

CARE! The medicinal paraffin becomes very hot in this experiment.

CARE! Perform this experiment in a fume cupboard.

CARE! Eye protection must be worn.

What you do

1. Introduce about 0.50 cm³ of the oil sample into the 5 mm diameter tube.
2. Slide the melting point tube into the sample with the sealed end uppermost.
3. Attach the 5 mm tube to a thermometer, so that the bottom of the tube is next to the thermometer bulb.
4. Carefully clamp the arrangement so that it is held in the 100 cm³ beaker, containing 75 cm³ of medicinal paraffin.
5. Heat the beaker and its contents using an electric hotplate.

6. At first there will be a slow escape of bubbles from the open end of the melting point tube as the air inside expands. When the sample reaches its boiling point there is a rapid release of bubbles. Note the thermometer reading when this just occurs.

7. Repeat with the remaining samples of oil. Comment on the values you obtain. Limonene has a b.p. of 175-176 °C, which sample do you think is the most pure?
Activity 1 Limonene from citrus fruits

Part G: Thin layer chromatography analysis of the oil samples

In this experiment you will use thin layer chromatography to investigate the composition of the extracted citrus oils. The 20:80 %v/v glacial ethanoic acid:ethanol solvent highlights the polar components in the oils. The 90:10 %v/v hexane:glacial ethanoic acid solvent highlights the non-polar components.

Requirements

Samples of the citrus oil extracts
10 cm$^2$ 20:80 %v/v glacial ethanoic acid:ethanol solvent A
10 cm$^2$ 90:10 %v/v 60-80 petroleum:glacial ethanoic acid solvent B
t.l.c. plates
250 cm$^3$ beaker
500 cm$^3$ beaker containing iodine crystals and sealed with cling film

Safety

CARE! Eye protection must be worn.
CARE! Must evaporate only in a fume cupboard.
CARE! Iodine solid is corrosive and harmful if left on skin for sometime.

What you do

1. Take a small t.l.c. plate and place one spot of the sample about 1.5 cm from the bottom of the plate. Use a melting point tube and keep the spot below 3 mm in diameter.
2. Run the chromatogram in a covered beaker, using solvent A.
3. When ready, remove the chromatogram and allow the solvent to evaporate in a fume cupboard. It is important that you do this thoroughly. If the plate is still wet you will notice the odour of ethanoic acid remains.
4. Locate the spots, corresponding to the components in the sample, by placing the plate in a beaker containing a few crystals of iodine. Cover this with cling film and leave for several minutes.
5. Sketch the appearance of the plate after you have located the spots.

Repeat with solvent B and with the remaining samples of oil. Which sample appears most pure? Does your answer agree with your conclusions from the determination of boiling point?
Activity 2 Chemical reactions of limonene

In this activity you can investigate some of the chemical characteristics of limonene. You will probably not have extracted enough citrus oil to perform all of these chemical tests. If you need to you may use limonene from a chemical supplier. The cheaper sources will contain both the d- and l- isomers of limonene, each with its own odour characteristics. You can also buy essential orange oil from some pharmacists as an aromatherapy ingredient.

Requirements
- Sample of limonene or extracted oil
- 3 x test tubes
- Test tube rack
- Teat pipette
- Mixture of 0.005 moldm$^{-3}$ potassium manganate(VII) and 2 moldm$^{-3}$ sulphuric acid (1:1 by volume)
- Concentrated sulphuric acid
- Approximately 0.5M bromine water

Safety
- Eye protection must be worn.
- Limonene is flammable
- Acidified potassium manganate(VII) solution is an irritant
- Concentrated sulphuric acid is corrosive
- Bromine water is toxic and corrosive

What you do

Reaction with acidified potassium manganate(VII)
1. Use a teat pipette to transfer about 0.5 cm$^3$ of limonene into a test tube.
2. Add five drops of the acidified potassium manganate(VII) and shake the tube until there is no further colour change.
3. Record the colour change and deduce the reaction that has occurred involving the C=C bonds in the limonene.
4. Carefully smell the contents of the tube. How is the odour different from limonene?

Reaction with concentrated sulphuric acid
1. Use a teat pipette to transfer about 0.5 cm$^3$ of limonene into a test tube. Add five drops of concentrated sulphuric acid and carefully shake the tube until there is no further change. Do not attempt to smell the product at this stage.
2. Put 2 cm$^3$ water into a large test tube (a boiling tube). Carefully add to this the mixture of limonene and concentrated sulphuric acid.
3. Record the changes and deduce the reactions that have occurred involving the C=C bonds in the limonene.
4. Carefully smell the contents of the tube. How is the odour different from limonene?

Reaction with bromine water
1. Use a teat pipette to transfer about 0.5 cm$^3$ of limonene into a test tube. Add five drops of dilute bromine water and shake the tube until there is no further colour change.
2. Record the colour change and deduce the reaction that has occurred involving the C=C bonds in the limonene. Do not smell the product.
Activity 3 Investigating the chemical stability of limonene

You will probably not have extracted enough citrus oil to perform all of these chemical tests. If you need to you may use limonene from a chemical supplier. The cheaper sources will contain both the d- and l- isomers of limonene, each with its own odour characteristics. You can also buy essential orange oil from some chemists as an aromatherapy ingredient.

Limonene molecules contain two C=C bonds and each of these behave in the same way as the double bond in ethene. This makes samples of citrus oil likely to change chemically, for the worse, during storage due to oxidation and polymerisation reactions.

In this activity you can investigate the factors that encourage these reactions to occur. By using the techniques described in Activity 1 (Parts E to G) and Activity 2 you can investigate how and how quickly the chemical, physical and odour properties of limonene change during storage.

You will need to compare a fresh sample of limonene with one that has been exposed to conditions that encourage oxidation and polymerisation.

Safety

In addition to the precautions listed in Activity 1 (Part G) and Activity 2 take care additional care since the air oxidation of limonene produces allergens. Wear plastic gloves to avoid skin contact.

Safety Part G

CARE! The medicinal paraffin becomes very hot in this experiment.
CARE! Perform this experiment in a fume cupboard.
CARE! Eye protection must be worn.

Safety for Activity 2

CARE! Eye protection must be worn.
CARE! Limonene is flammable
CARE! Acidified potassium manganate(VII) solution is an irritant
CARE! Concentrated sulphuric acid is corrosive
CARE! Bromine water is toxic and corrosive

Factors that encourage deterioration of citrus oil:
• light
• exposure to air
• transition metal ions
• heat

What you do

Keep the fresh sample in a dark glass bottle in a refrigerator. As a starting point you can perform an 'accelerated storage test' by leaving approximately 30 cm$^3$ of limonene in a 500 cm$^3$ conical flask, loosely plugged with cotton wool and left exposed to sunlight. At regular intervals over the following two weeks you can perform the tests, given in Activity 1 (Part E, Part F and G) and Activity 2 to detect how the sample has changed. In addition you can get an idea of the viscosity of the sample by pouring some into a test tube and stoppering it so that a small air space is left at the top. When you turn the tube upside down the time it takes for the bubble to rise to the surface is an indicator of the viscosity – the longer it takes, the more viscous the sample.
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**Interpretation of the results**

| Increase in boiling point | • Increased levels of impurities in the limonene  
|                          | • Polymerisation  
| Greater number of spots on the developed t.l.c. plate | • Oxidation reactions producing new components  
| Decreased tendency to react with manganate(VII), concentrated sulphuric acid and bromine water | • Reduction in the number of unsaturated molecules due to oxidation and/or polymerisation  
| Increase in viscosity | • Polymerisation  
| Increase in odour intensity | • Formation of oxygenates (e.g. carvone, limonene oxide  

Once you have established how quickly the properties of the limonene sample change under these conditions you can investigate the effect of varying each factor in turn.
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Limonene from citrus fruits

Part A
The volume of oil obtained in step 1 is quite small. It is important that students realise they are adding solvent to increase the volume of the oily layer so it can be separated more easily. The solvent is not extracting material directly from the grated rind.

Part B
Patience will be rewarded here. About 30 minutes is needed for an effective extraction.

Parts A, B and C
The solvent evaporates quite readily in the final step. Ensure that students follow the safety warnings.

Part D
Yields will vary greatly here, dependent on students' technique. On an industrial scale, economic factors need consideration - energy costs, cost of solvents, cost of maintaining the plant, labour costs.

Part E
The citrus top note will predominate here.

Part F
Pure limonene has a b.p. of 175-176 ºC. The citrus oil extracted is not pure limonene, expect experimental values above 176 ºC.

Part G
Solvent extraction produces a coloured sample with many more 'spots' than the samples from expression and steam distillation. It therefore produces an extract with more components (including pigments from the rind). Students may find this extract has the highest b.p.

Activity 2
The observations should be typical of those obtained from an alkene. Both manganate(VII) and bromine water are decolourised. Concentrated sulphuric acid, followed by water produces an alcohol that dissolves reasonably well in water. Students may find that treatment with manganate(VII) and concentrated sulphuric acid/water produce materials with greater odour intensity and harshness.

Activity 3
Ensure that students follow the safety warnings. Students are free to plan their investigation. It is essential that you regularly monitor their plans.