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Oranges and Lemons

Experiments in which students learn about the chemistry of limonene using simple equipment made from yoghurt pots, pans, 3D cinema glasses and a homemade colorimeter

Keywords: chiral molecule, limonene, steam distillation, polarimeter, colorimeter

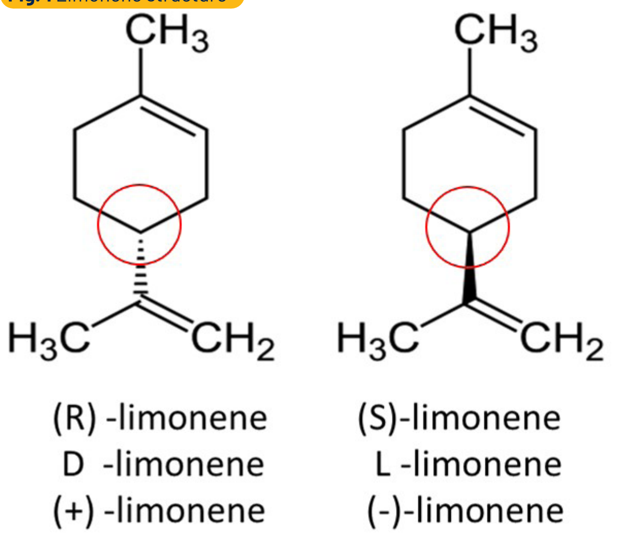
Disciplines: chemistry, physics, biology

Age level of students: 14–18

Introduction

The Mediterranean is permeated by the perfumes of fragrant plants. Oranges and lemons hold a special place in the cultures and cuisines of several countries and both pines and citrus fruits contain limonene.
Limonene is a chiral terpene derived from two units of isoprene (2-methyl-1,3-butadiene) which is responsible for the odour of lemons, oranges and pine. It exists in two forms, R and S, the only difference between the two being the relative positions of the bonds around one of the carbon atoms in the molecule: one form is the mirror image of the other (Figure 1). We perceive this difference in their odours because one form smells of lemons/oranges while the other smells like camphor. We can measure this difference in the way the two forms interact with polarized light. Limonene also contains two double bonds, so we can estimate the concentration of limonene in a sample using a simple colorimetric reaction.

Fig. 1 Limonene structure



What the students do

This project offers students a series of tasks to solve using simple equipment.

1. Extraction of limonene from the exocarp, the tough outer skin of lemons and oranges by steam distillation.
2. Characterization of limonene as a terpene by an addition reaction leading to a colorimetric readout and determination of its concentration in the distillate with a home-built colorimeter.
3. Verification of the optical activity of limonene with a home-built polarimeter.

1. EXTRACTION OF LIMONENE

The aim of this experiment is to demonstrate the extraction of a volatile organic compound using steam distillation. Limonene boils at 176 °C at which temperature it also decomposes. By boiling the citrus zest with water and condensing the vapour, we can isolate limonene at a much lower temperature without decomposing it. The limonene appears as a colourless oily liquid on the surface of the water collected during the distillation.

MATERIALS

- tall pan (an asparagus cooker with a basket was ideal for this)
- lid with a central hole
- beaker for ice-water
- 100 ml conical flask to collect the extract
- rubber piping
- duct tape
- stoppers
- lemon or orange zest
- heating plate

Fig. 2 Steam destillation of lemon zest

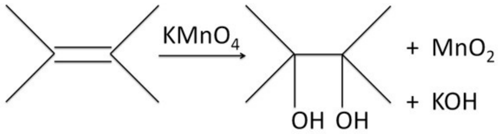
METHOD

Remove the zest (the yellow part of the skin) from 6 lemons, place it in the basket and cover it with water. Connect the rubber tube to the pot lid, ensuring a good seal and pass it to the collection flask which should be placed in the container with ice-water. Bring the water to the boil and check for losses of steam from between the pot and the lid.

(Safety: the whole system is at atmospheric pressure because it remains open, therefore there should be no danger from pressure build-up, however, beware of the hot surfaces!)

As the distillation proceeds, you will see water collecting with an oily patina on its surface. This is a mixture of limonene and water. You will also notice a very strong smell of lemons!

Fig 3 Reaction of double bond with permanganate



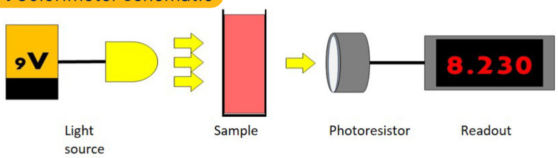
2. CHARACTERIZATION OF LIMONENE AS A TERPENE

Limonene contains two double bonds which react with potassium permanganate in a reaction associated with Alfred von Baeyer (Figure 3). The intensity of the purple solution decreases as it reacts with limonene. Therefore, this reaction can be used to estimate the amount of limonene in the extract by using a simple colorimeter.

2.1. BUILDING A SIMPLE COLORIMETER

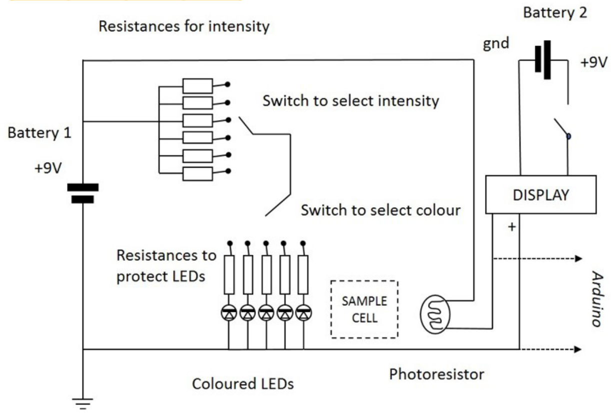
A colorimeter allows you to find the quantity of a coloured substance in a solution by measuring how much light of a particular wavelength is absorbed by the sample (Figure 4).

Fig 4 Colorimeter schematic



Professionally, this is done with a spectrophotometer. However, it is possible to build a colorimeter that works at a single wavelength using a simple circuit diagram (Figure 5) which allows us to change the wavelength and intensity of the light source.

Fig 5 Circuit for colorimeter



The principle behind the colorimeter is the Beer-Lambert law, which states that the amount of light absorbed by a solution of a substance is proportional to the concentration[1]. By preparing a series of permanganate solutions of known concentration and measuring the voltage across the photoresistor for each, we are able to draw a standard curve which shows the relationship between the voltage on the *y*-axis and the concentration on the *x*-axis (Figure 6). Remember to subtract the value of the water blank from the values for the samples. The concentration of limonene in the distillate is estimated by working out how much permanganate has been consumed during the reaction with the limonene.

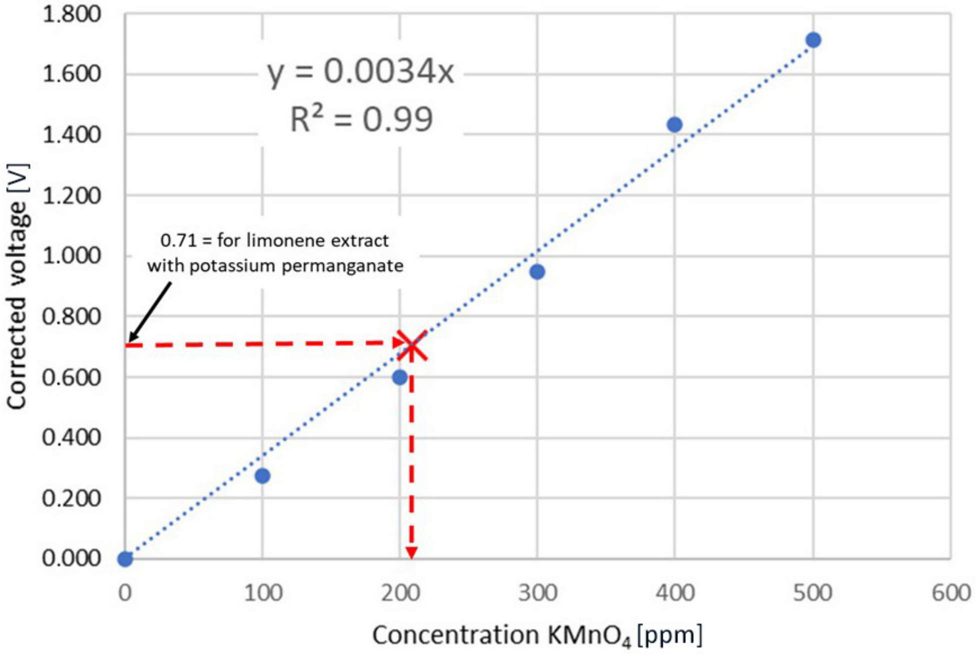
MATERIALS

- colorimeter
- 5 or 6 plastic cuvettes
- measuring cylinders
- droppers
- solution of $KMnO_4$ (500 ppm = 500 mg in 1000 ml) diluted to 400, 300, 200 and 100 ppm
- 1M H_2SO_4

Table Typical values for standard soluttions

Concentration of potassium permanganate [ppm]	Voltage measured (average of 3 measurements) [V]	$Voltage_{H_2O}$ - Voltage [V]
0	8.503	0.000
100	8.229	0.274
200	7.901	0.602
300	7.554	0.949
400	7.071	1.432
500	6.791	1.712

Fig 6 Standard curve for potassium permanganate



You should do a standard curve every time you use the instrument and the exact voltages you obtain will depend on the electronic components used.

We drew a dispersion graph of voltage versus concentration in Excel and calculated a trendline for the linear relationship. The high value for the regression coefficient (R^2) tells us how good our trendline is. Values close to 1.0 tell us that the line is a good description of the data.

METHOD

Shake the extract vigorously and take a 5 ml sample, add distilled water to bring the total volume to 100 ml. Take 5ml of this solution and add 4 ml of 400 ppm potassium permanganate followed by 1 ml of 1M H_2SO_4 then leave for 30 minutes at room temperature. Read the value of the solution in the colorimeter and repeat. We did 12 separate readings to obtain a mean of 0.71 V corrected for the water blank.

We assume that the limonene reacts completely with the permanganate and the amount consumed can be estimated by measuring the amount or permanganate remaining at the end of the reaction.

The corresponding concentration of potassium permanganate can be read directly from the graph (the red dotted line on figure 6) or we can compute it from the formula of the best-fit line calculated by Excel. In this case the formula is:

$y\,[\mathrm{V}] = 0.0034x\,[\mathrm{ppm}]$

Our concentration was estimated as

$x = 0.71/0.0034$ ppm ≈ 208 ppm,

hence, the permanganate used was

≈ 400 ppm - 208 ppm ≈192ppm.

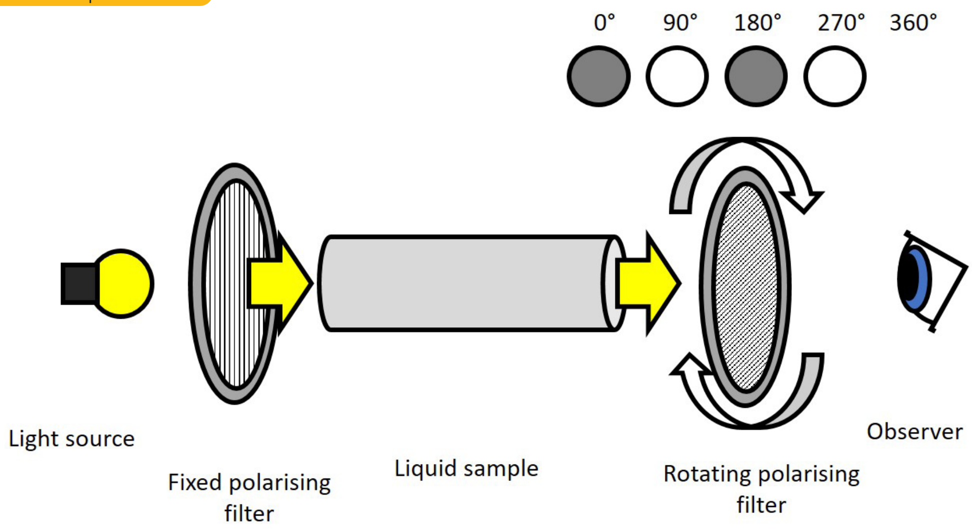
From the structure of limonene, this is equal to twice the concentration of double bonds, hence the amount of limonene is ≈ 91 ppm, or about 0.1 g/l. This compares with values reported in the literature[2].

The change in colour can be clearly demonstrated by using a large excess of limonene, but for the distillate, students may not be able to discern a colour change by eye. The change in the concentration of the permanganate can be observed by using the colorimeter to measure the voltage. The graphing method introduces students to the concept of making a standard curve to measure the concentration of an unknown solution.

3. VERIFYING THE OPTICAL ACTIVITY OF LIMONENE

Didactically, chirality in organic molecules can be very confusing because it requires a familiarity with drawing structures combined with abstract spatial thinking. The rotation of polarised light by an enantiomerically pure substance needs to be demonstrated since this effect cannot be predicted by simply looking at a drawn structure. This can be done using a polarimeter made up of a

Fig 7 Schematic of a polarimeter



light source, two polarizing filters (one fixed, the other rotatable) and a sample tube between the two filters (Figure 7). Working in groups, students build their own polarimeters and they are encouraged to explore different designs to solve the main technical problems, i.e. to reliably rotate the polarizing filters around the optical axis and to get a good extinction of the light (Figure 8).

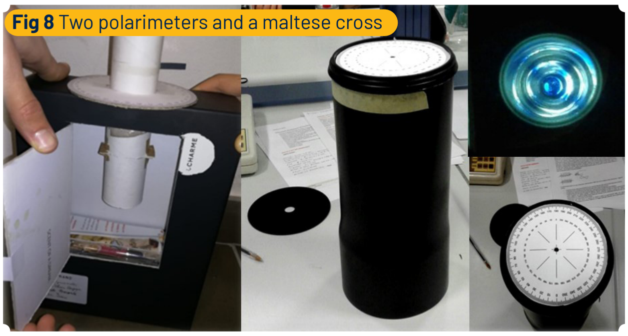


Fig 8 Two polarimeters and a maltese cross

The amount of rotation observed for a sample depends on the amount of compound the polarized light encounters as it passes through the sample. The rotation varies according to concentration and the length of the light path (i.e. the height of the sample in the tube). More concentrated solutions and longer path lengths give larger rotations.

MATERIALS

- a container to hold the sample and the filters
- two polarizing filters (e.g. 3D cinema glasses)
- a sample tube
- a light source
- a protractor to measure the angle
- 100 % enantiomerically pure limonene as a standard

THE YOGHURT POT POLARIMETER (A SIMPLE VERSION)

Take a clean 1 kg yoghurt pot or equivalent (Figure 9). Find the centre of the bottom of the pot, remove a small square of plastic from around it and cover the hole with a polarizing filter. Identify the centre of the lid and cut a small square around it, then attach the second polarizing filter. Check that the lid with the filter rotates freely. Shine the light source through the bottom filter and rotate the lid to change the intensity of the light as the filters go in and out of alignment. If the effect is not very visible, reduce the intensity of the light source. Attach a protractor to the lid to measure the angle of rotation. Black paper inside the yoghurt pot reduces the effect of incident light.

HOW TO USE IT

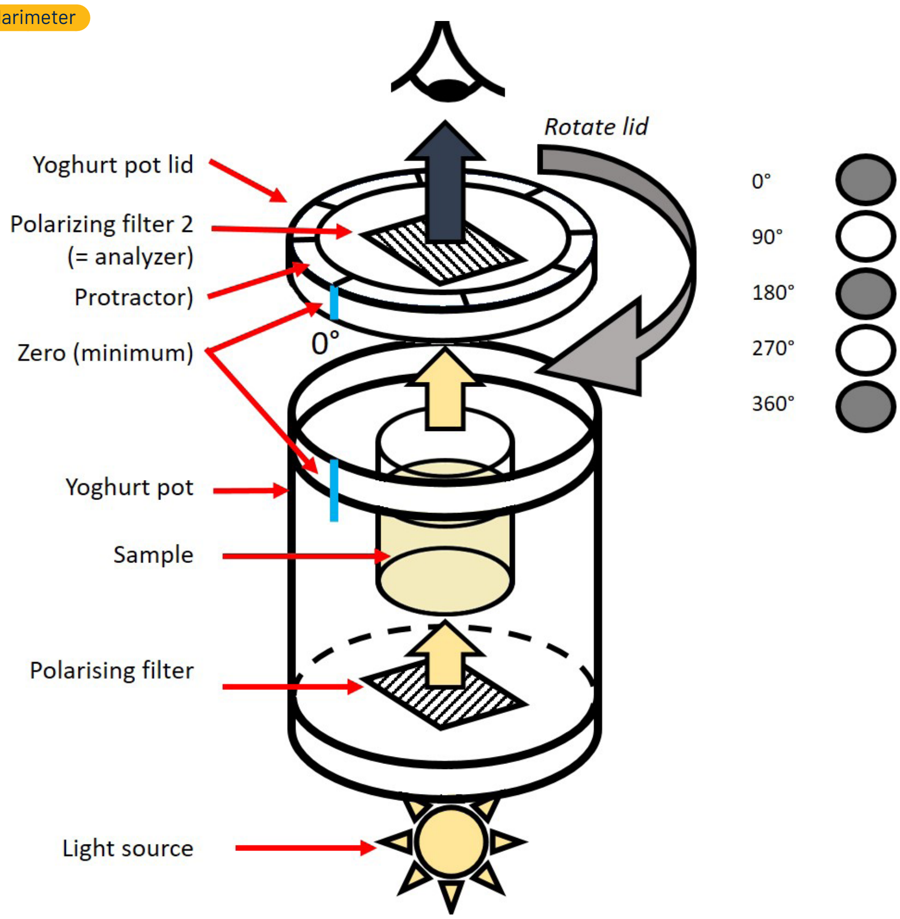
Place a tube with 10 cm height of water inside the pot between the filters so you can see the light shining through it. Keeping the instrument steady, look down through the sample to the light source and rotate the lid until the image is as dark as possible. Make a note of this position, which is the zero point of the lid with respect to the body of the instrument.).

Change the water for pure limonene (10 cm height) and observe that the light is now visible at the zero point. Rotate the lid clockwise until the light is no longer visible. The angle the lid is rotated is the extinction angle for that pathlength of sample at that concentration.

WHAT YOU OBSERVE

Using white light, it is likely that you will observe dispersion of colours (birefringence) upon rotating the filter

Fig 9 Yoghurt pot polarimeter



and the Maltese cross pattern near the extinction point (upper right in figure 8). This is a curious phenomenon in itself which can lead to a discussion of polarization in crystallography. Here, this interferes with the reading and can be reduced by changing to monochromatic light source by using a filter. Students can also observe the effect of the wavelength of light on the rotation angle by using different coloured filters.

TYPICAL MEASUREMENTS

The amount of limonene extracted is extremely small and it is not technically possible to observe its optical rotation with such a crude instrument. However, both forms of limonene are available as standards: (R)-limonene is used in furniture restoration and the (S)-form can be purchased from a chemical supplier.

Students measure the angle of rotation as a function of the height of limonene in the tube. They then use the following relationship to estimate the standard optical rotation of pure limonene:

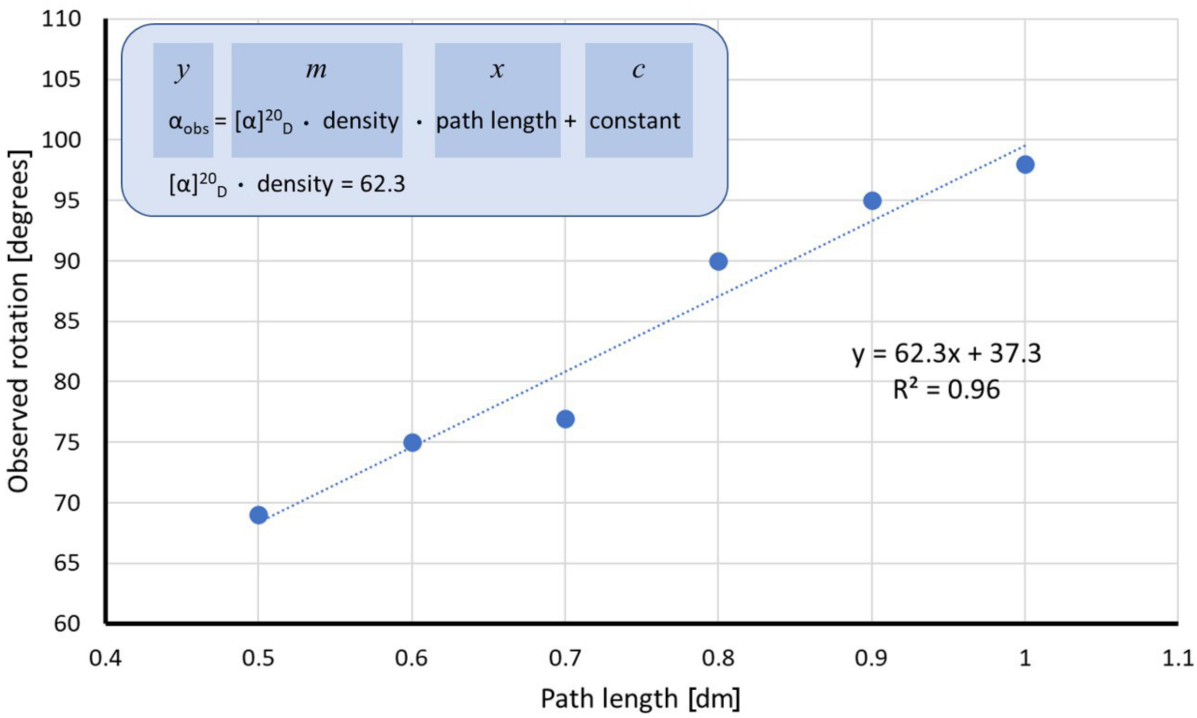
$$\alpha_{obs} = [\alpha]^{20}_{D} \cdot \text{density}\,[\text{g cm}^{-3}] \cdot \text{path length}\,[\text{dm}]$$

Here, α_{obs} is the angle measured in the experiment and we need to find $[\alpha]^{20}_{D}$ which is the value reported in the literature at 20 °C, using a sodium lamp. We approximated the sodium lamp with an orange filter and the laboratory temperature was 18 °C. By changing the path length, we obtained several values of α_{obs} which allowed us to draw a graph (Figure 10). This is a straight line with a gradient of $[\alpha]^{20}_{D} \cdot$ density (= 0.84 g cm^{-3} for limonene). We estimated $[\alpha]^{20}_{D}$ as 74° which is low compared to the literature[3]. However, since the observed rotation is very sensitive to wavelength and to temperature as well as other sources of error in the experiment, this presents a challenge to students in how to improve the measurement.

A final note and some further explorations

Having samples of both enantiomeric forms in hand allows students to compare the odours of the two compounds. While (R)-limonene has a distinct citrus odour, (S)-limonene smells of camphor and the two cannot be confused. This is a clear demonstration of the chirality of our olfactory receptors!

Fig. 10 Variation of angle of rotation of pure limonene with pathlength



The polarimeter can be used to study the optical rotation of solutions of easily available sugars such as sucrose and fructose which have opposite rotations that are sufficiently large to be easily measured with these simple instruments. The acid catalysed hydrolysis of sucrose to give a mixture of fructose and glucose (also known as inverted sugar), is easy to follow over a short time period in an impressive yet simple demonstration of optical rotation[4].

Acknowledgements

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References

[1] Stewart J. Tavener and Jane E. Thomas-Oates, Build your own spectrophotometer, accessed at www.rsc.org/images/EiC-05_2007-spectrophotometer_tcm18-214788.pdf

[2] Ahmed Saleh, Extraction of the Essential Oil Limonene from Oranges, accessed at https://natural-ingredient.org/wp/wp-content/uploads/Limonene.pdf

[3] values can be consulted in the Sigma-Aldrich catalogue

[4] hydrolysis of sucrose accessed at www.colby.edu/chemistry/PChem/lab/InversionSucrose.pdf

All illustrations, pictures and graphs were created by the authors.

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