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Citrus science: learn with limonene

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When life gives you lemons: use limonene to explore molecular properties with your students and show them the scientific method in action.

The activities in this article allow students to investigate simple molecular properties using an easily accessible compound: limonene. The activities were developed by groups of Italian and Spanish students in a joint project through written assignments, questions, and videos.

Through the application of the scientific method, students develop the ability to observe a phenomenon, formulate a hypothesis, search for suitable materials to set up an experiment, collect data for analysis, and arrive at a conclusion that is shared with others. The focus here is on a simple organic molecule, (*R*)-(+)-4- and (*S*)-(–)-4-isopropenyl-1-methyl-1-cyclohexene (the two enantiomers of limonene), which is safe, easy to find, and of real-world and industrial interest.

Experiments:

- optical properties of (*R*)- and (*S*)-limonene (2 h)
- latex dissolution by limonene (1 h)
- nonpolarity of limonene (1 h)
- differences in smell between (*R*)- and (*S*)-limonene (1–2 h)

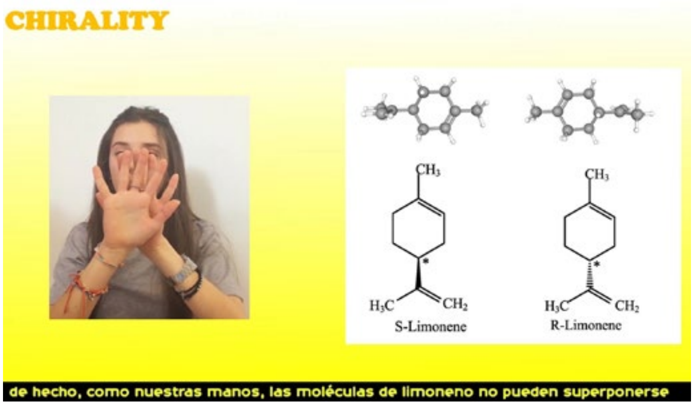
Extension activities: the antibacterial and anti-germination properties of limonene (2 h)

Safety notes

Limonene is a volatile terpene, which, if concentrated, must not be swallowed or come into contact with the eyes because it is an irritant. It must be immediately washed from the skin. Unlike in orange peel, the limonene content in resin and turpentine does not reach critical concentrations.

Activity 1: Understanding optical properties

In the first activity, we discuss chirality using (*R*)- and (*S*)-limonene. This activity should take around 60 min.



Structures of (*S*)- and (*R*)-limonene; like our hands, these enantiomers are mirror images and not superimposable. The chiral centre is indicated with *.

Image courtesy of the authors

Materials

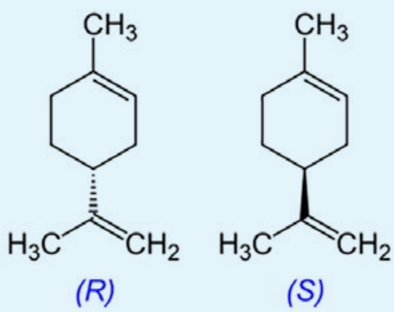
- Resources on limonene and isomers
- ball-and-stick molecular models
- sheet of paper and poster (tempera) paints

Procedure

1. Introduce limonene and enantiomers using the infosheet, textbooks, and the resources provided for this article.
2. Students should fold a sheet of paper in half and draw the structural formula of (*R*)-limonene, with all atoms and bonds, on one half.
3. Students should then identify the chiral carbon (C*), sp^3 hybridization, and tetrahedral structure of the compound. They should then draw the enantiomer with respect to C*.

Limonene

Limonene (4-isopropenyl-1-methylcyclohexene) is a chiral cyclic monoterpene, with the molecular formula $C_{10}H_{16}$.[1–3] This chiral compound exists in two isomeric forms, which are stereoisomers; in other words, molecules with the same structure but with different orientations of the same atoms in space. The two forms of limonene differ only in the configuration of the groups around a single carbon atom. The two forms are mirror images (=enantiomers) of each other that can only be interconverted by breaking and forming covalent bonds.



Molecular Formula	$C_{10}H_{16}$
Molecular Weight	136.23
Synonyms	D-Limonene, (*R*)-(+)-Limonene L-Limonene, (*S*)- (–)-Limonene

D-Limonene is a major component of the oil in citrus peels, which also contains citrus limonoids.[4] The less common L-limonene is found in herbs like caraway. Industrial limonene comes from the peel of citrus fruits as a waste product of fruit processing for the production of juices.[5] For this reason, it is produced as the *R* enantiomer (D-limonene). It is used as a fragrance compound and for degreasing.

Like all enantiomers, the two forms are indistinguishable in most chemical/physical tests. They do, however, rotate the plane of polarized light in opposite directions. Thus, it is possible to distinguish the enantiomers by using a polarimeter.

Furthermore, they are easily distinguished biologically. D-limonene (the *R* form) has a sweet citrus odour, while the L form has a has a sharper resinous or turpentine-like odour. Our noses are very sensitive and able to distinguish these very similar compounds because the olfactory receptors in the nose have molecular sites that interact specifically with different enantiomers.[6]

4. Students can then take a new A4 sheet and fold it in half. On one half, they should draw the chiral centre with the four bonds in perspective. Next, they should use a finger to dab a different colour of poster paint at the ends of the bond lines. They then fold the sheet to transfer the paint dabs to the other side, and redraw the bond lines. This helps to illustrate how enantiomers are mirror images.
5. Build limonene with the ball-and-stick models and identify C*.

Activity 2: Investigating properties of (*R*)- and (*S*)-limonene

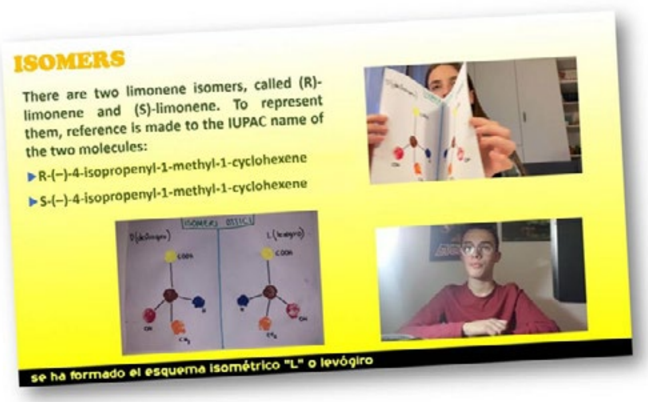
Next, students can investigate chirality using (*R*)- and (*S*)-limonene and see how this very minor structural change can have real-world consequences. It should take around 60 min.

Materials

- polarimeter (optional)
- 1 ml (*R*)- and (*S*)-limonene (98–100%) (if you have a polarimeter)
- peel from fresh oranges and lemons
- Vaseline or similar unscented petroleum jelly in small containers

Procedure

1. Place the pure (*R*)- and (*S*)-limonene in cuvettes or the tubes required for your polarimeter, and let the students record the characteristic angle of rotation of limonene with polarized light, as well as the extinction coefficients of both enantiomers.
2. Mix a small drop of each enantiomer into petroleum jelly and pass the samples round to see if students can differentiate them.
3. Give students strips of lemon and orange peel to smell and ask them which enantiomer they think the peels contain.



Exploring chirality with coloured paint

Image courtesy of the authors

Discussion

The specific rotation angle to recognize each enantiomer is +/− 123°, but, in reality, it is sufficient to observe that the *R* enantiomer is extinguished by rotating the analyzer to the right, while, for the *S* enantiomer, rotation is in the opposite direction with the same angle.

The smell of citrus also depends on the citrus limonoid molecules. We have added references and a theoretical activity to understand the difference between these molecules and study the metabolic pathways in plants.

Optional extension activity

The perception of the smell of (*R*)-limonene as being different from that of (*S*)-limonene is common but not universal. Students could design an experiment to test this, e.g., to determine the percentage of the population in a sample group able to distinguish them.

Activity 3: Latex dissolution by limonene

Next, students can investigate the effect of limonene on different polymers. This activity should take around 1–2 h. All steps should be performed by the students. There are quite a lot of tests, so, if necessary, students can work in groups to save time/equipment.

Materials

- 1 ml (*R*)- and (*S*)-limonene (98–100%)
- peel from fresh oranges and lemons (2 of each)
- children's balloons in latex, mylar, or polyester plastics
- rubber and polyvinyl gloves
- ethylene shopping bags
- latex condoms
- plastic pipettes
- 10 cm embroidery hoops or small beakers
- elastic bands

Procedure

1. Cut the balloons, gloves, and bags and stretch them over the embroidery hoop or beaker like the skin on a drum. If using beakers, secure them with elastic.

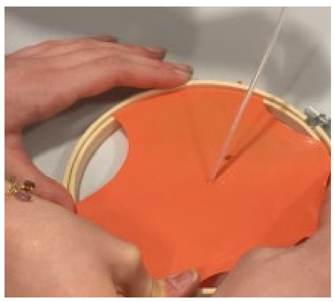


Image courtesy of the authors

Dissolution of latex in a balloon and condom

Image courtesy of the authors

2. Ideally, two samples of each should be prepared to allow the experiment to be duplicated.
3. Cut a 2 cm x 2 cm piece of citrus peel and bend it to spray oil onto a clean finger. Touch the sample under tension, or place the peel directly on the sample, and note the results.
4. Repeat the operation with (*R*)- and (*S*)-limonene, dropping a small drop onto the sample under tension with a pipette.
5. For inflated balloons and condoms, it is possible to touch them with a clean finger and then with a finger coated with limonene and observe the explosion.
   If working in groups to cover all the samples in the previous steps, all students should get the opportunity to try this one.

Discussion

Latex material, made up of 30–40% cross-linked polyisoprene, reacts with limonene, 'opening' a hole around the drop or touch point. This is because the limonene molecules (monoterpenes) are small enough to slip along the polyisoprene chains and move them apart, loosening the chemical curing. This is enough to snag the sample and pop the gas-filled balloon. The reaction is proportional to the concentration of limonene and to the thickness of the rubber, but it does not differentiate between (*R*)- and (*S*)-limonene.

Activity 4: Nonpolarity of limonene molecule

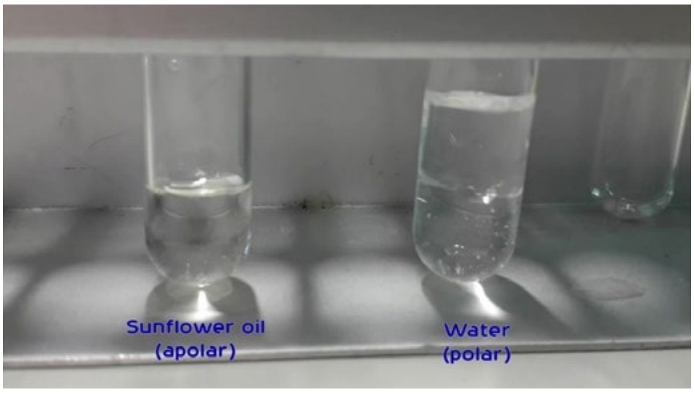
In this activity, we investigate the chemical properties of limonene. It should take around 30 min.

Materials

- 6 ml (*R*)- and (*S*)-limonene (around 100%)
- 3 ml corn or sunflower oil
- water
- 2 test tubes
- 4 plastic pipettes

Procedure

1. Put 3 ml of limonene in tube 1 and carefully add 1 ml of oil down the wall, without mixing.
2. Put 3 ml of limonene into tube 2 and carefully add 1 ml of water to the wall, without mixing.
3. Observe if there are any separating surfaces between liquids.
4. Limonene can be recovered from the water mixture by separation with a pipette for reuse in other experiments.



Limonene with water or oil. The levels in the two tubes are different because the students were a little inaccurate. Exact volumes aren't important.

Image courtesy of the authors

Discussion

Limonene is soluble in oil and mixes in it naturally: it is deduced that it is a nonpolar solute that dissolves in a nonpolar solvent.

Limonene is insoluble in water and does not mix with it: limonene is a nonpolar molecule that does not dissolve in water, a polar molecule.

The polarity of a molecule depends on the chemical species that form it, on the difference in electronegativity present in the covalent bonds, and on the fact that the centre of the positive electrostatic forces does not coincide with the negative ones.

Limonene is a hydrocarbon consisting of carbon and hydrogen atoms. It is a monoterpene, an isoprene derivative, with a closed chain in which the only areas with a weak partial negative charge are double bonds; these charges are too weak to result in an interaction with water molecules. «

Acknowledgements

This activity was presented at the Science on Stage Festival 2022

References

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[2] Perveen S (2018) Introductory Chapter. In Perveen S, Al-Taweel A (eds) *Terpenes and Terpenoids* pp 1–12. IntechOpen. ISBN: 978-1-78984-777-2

[3] Chemical information for limonene and a virtual 3D model: https://pubchem.ncbi.nlm.nih.gov/compound/Limonene#section=3D-Conformer

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[5] Jongedijk E et al. (2016) Biotechnological production of limonene in microorganisms. *Applied Microbiology and Biotechnology* **100**: 2927–2938. doi: 10.1007/s00253-016-7337-7

[6] Malnic B, Godfrey PA, Buck LB (2004) The human olfactory receptor gene family. *PNAS* **101**: 2584–2589. doi: 10.1073/pnas.0307882100. This article has a correction.

Resources

- Watch students from the Science on Stage joint project present their findings (video in Italian and Spanish with English subtitles).
- Isolate limonene and other fragrance compounds from plant material: Allan A, Worley B, Owen M (2018) Perfumes with a pop: aroma chemistry with essential oils. *Science in School* **44**: 40–46.
- Discover the biochemistry of bananas: Glardon S, Scheuber T (2018) Go bananas for biochemistry. *Science in School* 44: 28–33.
- Investigate the properties of so-called superfoods: Frerichs N, Ahmad S (2020) Are 'superfoods' really so super? *Science in School* **49**: 38–42.
- Explore food chemistry with mushrooms: Bunjes F et al. (2017) Natural experiments: chemistry with mushrooms. *Science in School* **42**: 36–41.
- Try some further chemistry experiments based on limonene (suitable for older students).

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