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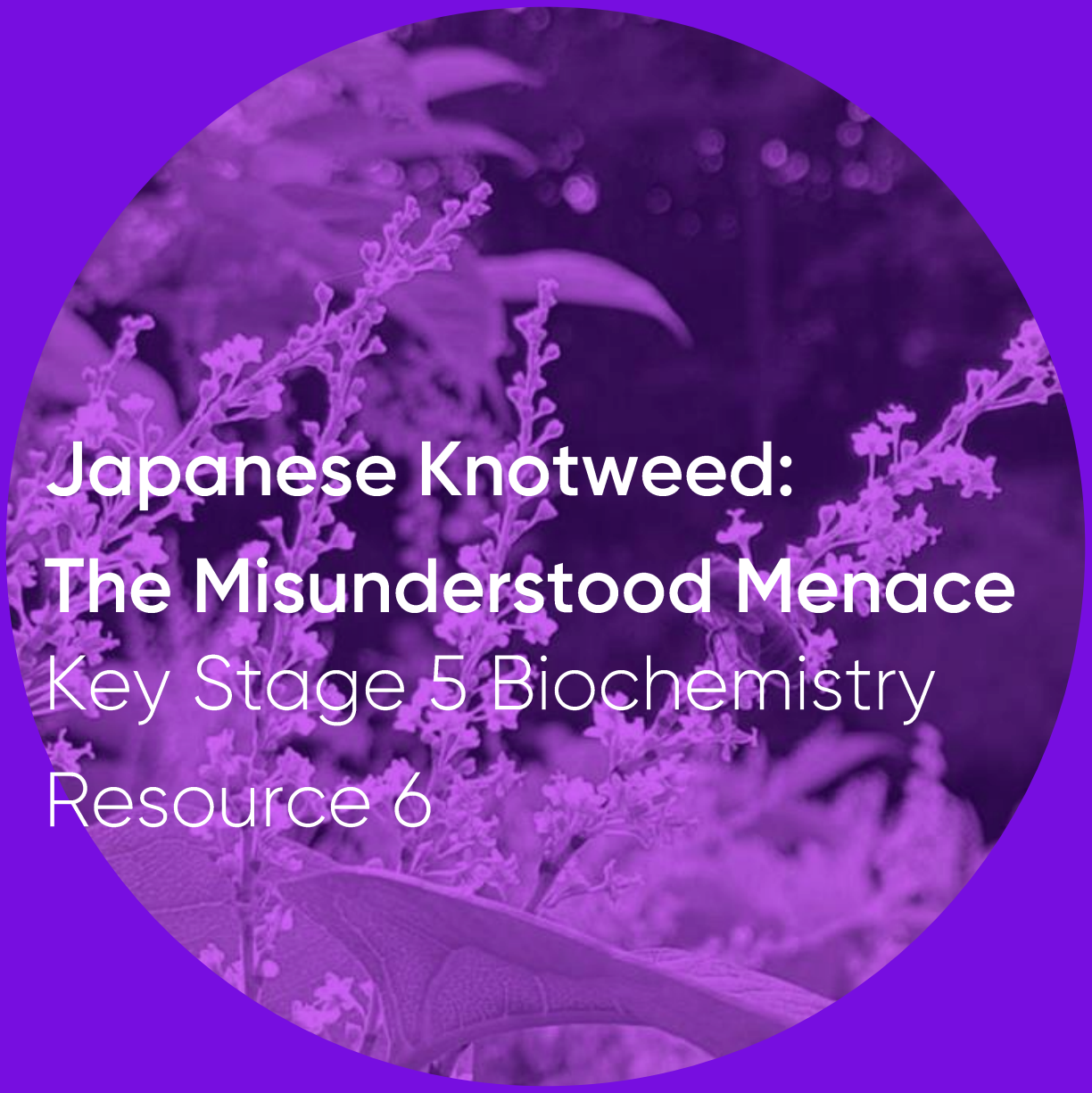

Japanese Knotweed: The Misunderstood Menace

Key Stage 5 Biochemistry

Resource 6

2019

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Resource Six Overview

Topic Information from Infrared

A-Level Modules Infrared spectroscopy

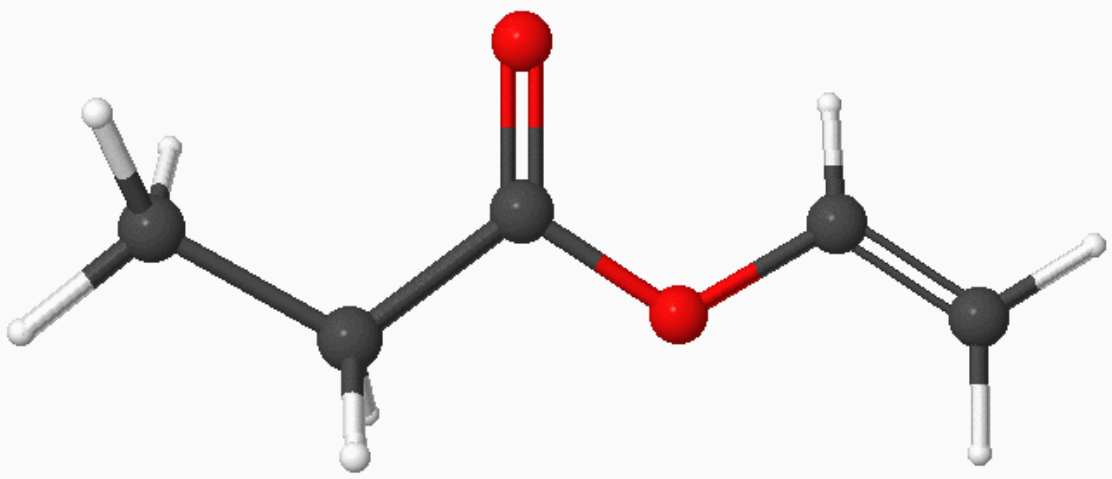
Objectives By the end of this resource, you will be able to understand:

- ✓ Bonds in a molecule absorb infrared radiation at characteristic wavenumbers.
- ✓ How 'fingerprinting' allows identification of a molecule by comparison of spectra
- ✓ How infrared spectroscopy can be applied to biology

Instructions

1. Read the data source
2. Complete the activities
3. Explore the further reading

Context When infrared light interacts with molecules, it causes them to change their vibrations. The bonds within the molecule bend and stretch in different ways. This resource explores how we can use the interaction between infrared light and molecules to find out more information about different samples. The use of infrared light in this way is known as infrared spectroscopy.



Resource Six
Data Source

Infrared spectroscopy is a technique used to identify different functional groups in a molecule. It is usually used by chemists to help identify a single molecule in a very pure sample. However, it can also be applied to more complex biological systems.

Section A

Molecular vibrations

Two atoms in a covalent bond are held together because both nuclei are attracted to the same pair of electrons. The two nuclei can vibrate backwards and forwards - towards and away from each other - around an average position. There are two basic modes of vibration – 'stretching' and 'bending'. Each bond vibrates at a different frequency. Vibration frequencies are affected by atom size, bond length and bond strength. Stronger bonds and lighter atoms vibrate faster.

Section B

Infra-red spectroscopy

In infrared spectroscopy, the energy from infrared light is absorbed by molecules in a sample, causing changes to their molecular vibrations. For a particular covalent bond in a molecule, only a particular set of vibrational frequencies is possible. Suppose a bond is vibrating at a frequency $\nu 1$ and its next available frequency is $\nu 2$; then, if radiation with a frequency ($\nu 2 - \nu 1$) is incident on the compound containing this bond, some of the radiation is absorbed and the bond vibrates at the higher frequency.

Determination of the frequencies in the infrared region which are absorbed by a compound gives information about the types of bonds which are present. The frequencies at which the energy was absorbed can be shown in an absorbance diagram, known as an infrared spectrum, which covers a range of frequencies, from around 4000 cm^{-1} to 500 cm^{-1}. The patterns produced in the spectra by IR absorptions are complex and only a few bond types can be identified, such as carbonyl groups C=O, and hydroxyl (alcohol) groups O-H.

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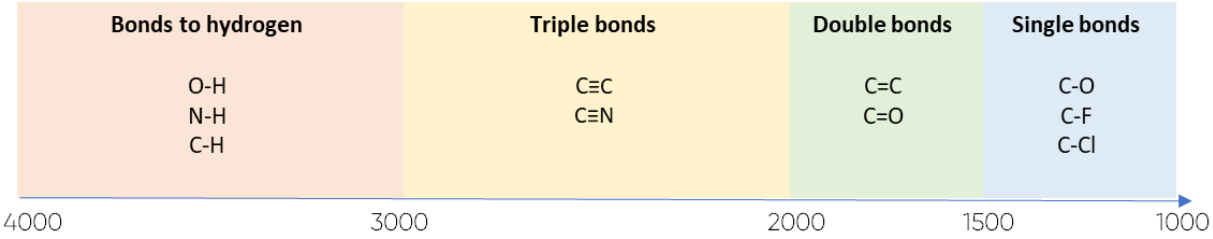
Data Source

Regions of the IR-spectrum

The Y axis of an IR spectrum is percentage transmission, and the X axis is wavenumber measured in cm-1.



Section C

The Fingerprint Region

The right-hand side of the diagram, from about 1500 to 500 cm-1, is known as the fingerprint region. This fingerprint region is too chaotic to pick out much information by eye. However, the fingerprint region is unique for each molecule and can be used for identification of specific molecules using pre-interpreted spectra from a database.

Section D

Intensity of absorption

IR spectroscopy gives information about the stretching and bending of bonds, particularly the kind of unsymmetrical bonds found in functional groups such as OH, C=O, NH2, and NO2. The intensity of an IR absorption increases with an increasing dipole moment as the bond is stretched. If two atoms in a bond have a very different electronegativity, then the bond will have a greater dipole moment, which will change more when it is stretched. This is why the intensity of absorption is higher for C=O functional group compared with a C-H bond. The oxygen atom is more electronegative than the carbon atom, and so holds a disproportionate amount of the electron density at that end of the bond. Hydrogen bonding can increase the width of the peaks, so O-H of alcohols are easily spotted by their broad peaks in the range 3230 – 3550 cm-1.

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Data Source

Section E

Application to Biosciences

As part of your exams, you will be asked to identify functional groups from the main regions of an example IR spectrum. This is something which organic chemists do frequently, as it helps them to check if they have made the right molecule. In organic synthesis chemistry, the aim is often to produce a pure sample containing only one type of compound.

In contrast, in biological systems, there are a multitude of molecules all mixed together. Imagine taking a spectrum of a cell in your own body and trying to interpret the vibrations caused by all the bonds in your DNA, the proteins in your enzymes, and the lipids in your cell membranes, all bending and stretching at once. Luckily, computer programs and coding can be used to apply maths to the spectra from complex samples.

A specific type of infrared spectroscopy called ATR-FTIR can be used to solve biological questions. ATR stands for Attenuated Total Reflectance, which is a type of sampling technique involving the passage of infrared light through a crystal. FTIR stands for Fourier Transform Infrared Spectroscopy. A Fourier transform is a mathematical process used to convert the raw data into the actual spectrum.

Rather than using the main spectrum to pick out functional groups, the computer plays spot the difference with the messy fingerprint region. Because of the individuality of the fingerprint region, minute changes in spectra can be seen. For example, the difference between the average spectra taken from a set of healthy cells can be compared to the average spectra from a set of ovarian cancer cells. Once the computer is trained to recognise the difference between healthy and cancerous cells, it can be used for patient diagnosis. This is an example of machine learning, where the computer uses a known fraction of a dataset to assign the rest of the data.

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Data Source

I am applying this technique to phylogenetics, the study of the evolutionary history and relationships among individuals or groups of organisms (e.g. species, or populations). Phylogenetics is traditionally worked out by looking at heritable traits, such as DNA sequences. The more similar two species are, the more closely related they are likely to be. I use ATR-FTIR and a mathematical analysis called hierarchical clustering to form linkage maps, similar to those achieved with genetic data. Unlike genetic analysis, ATR-FTIR does not require any long-winded sample preparation, DNA extraction, or sample destruction.

Resource Six Activities

Activities

Question 1

Bonds in a molecule _____ infrared radiation at characteristic wavenumbers. This causes a change to their molecular _____. Strong bonds vibrate _____ than weak bonds. Chemists use IR spectroscopy to identify the_____ _____in a molecule. '_____' allows identification of a molecule by comparison of spectra.

Question 2

Give me an example of how IR spectroscopy can be applied to Chemistry or Biology.

Question 3

Using the information in the following table, why does the bond C-Br vibrate more slowly than C-Cl?

ELEMENT	ELECTRONEGATIVITY	ATOMIC MASS/ U	BOND ENERGY OF C-X BOND/ KG MOL^{-1}
CHLORINE	3.16	35.453	428
BROMINE	2.96	79.904	362

Question 4

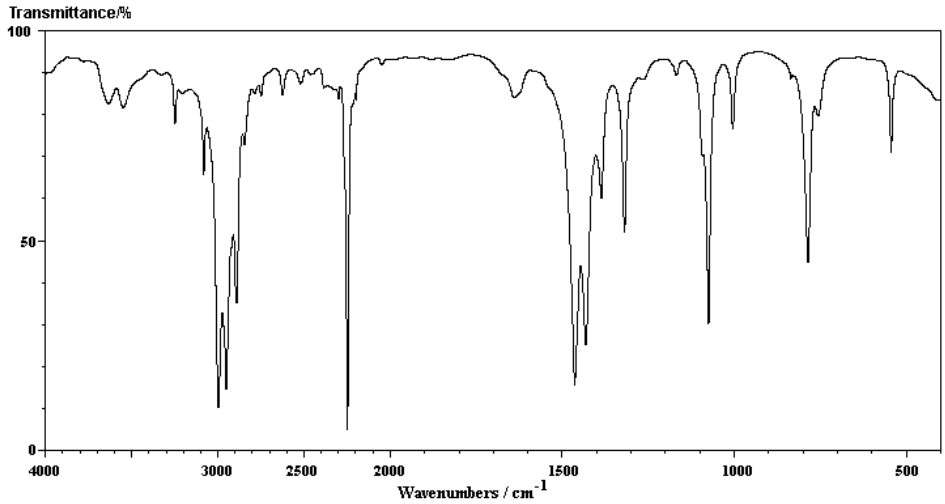
Problem solving

Using the infrared absorption data on the next page, match up the spectra A-D with the following molecules:

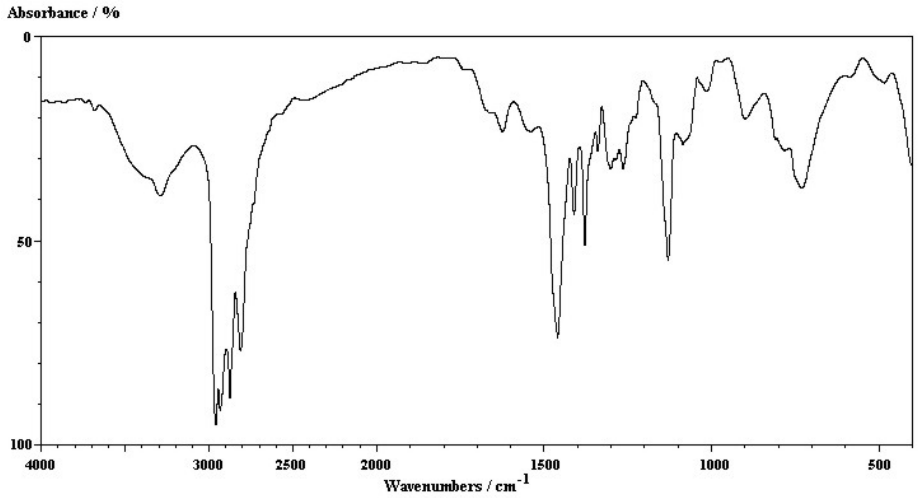
1. Butanal, CH3CH2CH2CH(=O)
2. Propionitrile, CH3CH2C≡N
3. Dodecane, CH3(CH2)10CH3
4. Dipropyl amine, (CH3CH2CH2)2NH

Resource Six Activities

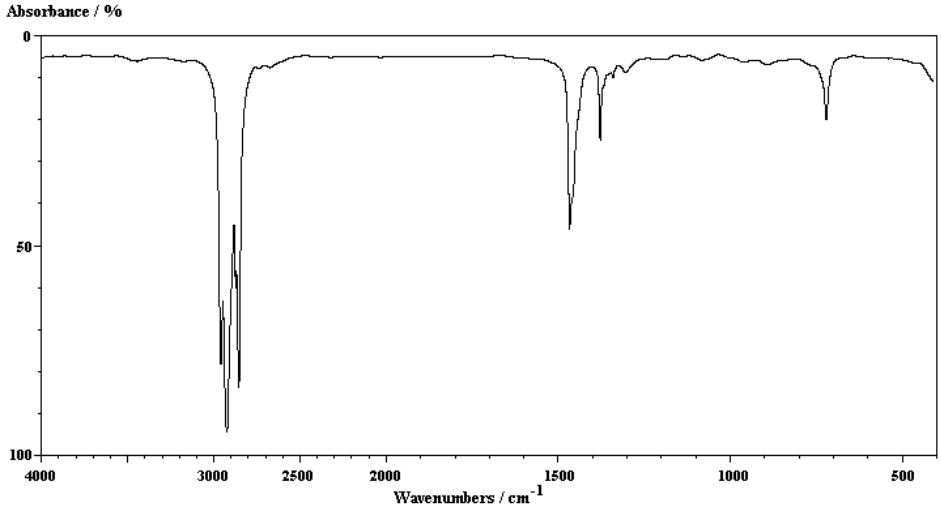
Spectrum A



Spectrum B

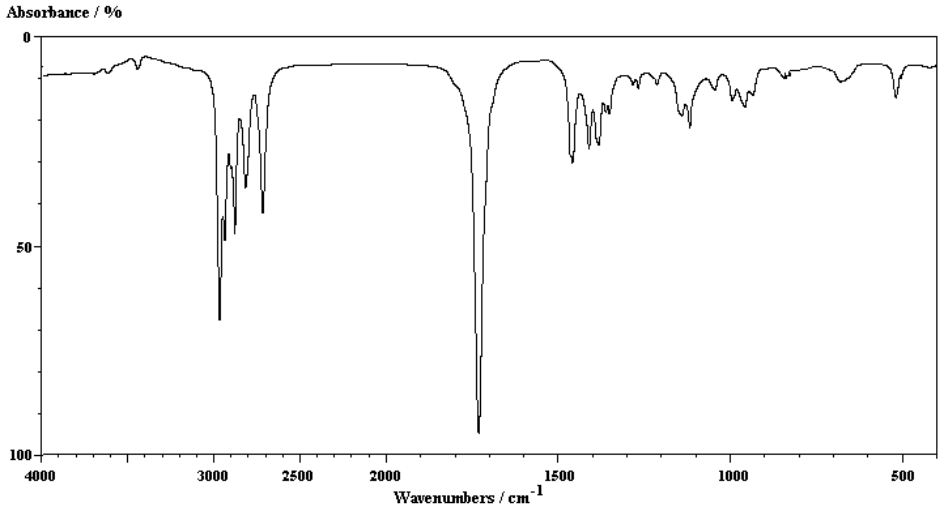


Spectrum C



Resource Six Activities

Spectrum D

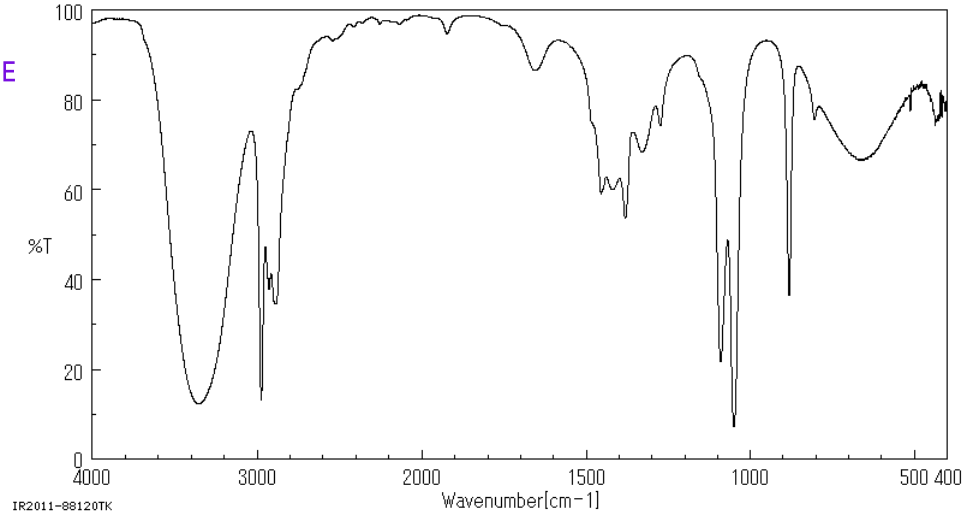


Question 5

Using the absorption table below, identify one feature of the infrared spectrum below (Spectrum E) of a pure sample of X that may be used to confirm that X is an alcohol.

Infrared absorption data	
Bond	Wavenumber/ cm^{-1}
N-H (amines)	3300 – 3500
O-H (alcohols)	3230 – 3550
C-H	2850 – 3300
O-H (acids)	2500 – 3000
C≡N	2220 – 2260
C=O	1680 – 1750
C=C	1620 – 1680
C-O	1000 – 1300
C-C	750 – 1100

Spectrum E



Resource Six
Activities

Question 6

The 'greenhouse effect' is a process that occurs when gases in Earth's atmosphere trap the Sun's heat. The greenhouse effect of a given gas is dependent on its concentration in the atmosphere and its ability to absorb infrared radiation. Using the table below, argue which of these three gases are greenhouse gases.

GAS	ABBREVIATION	BOND TYPE
Carbon Dioxide	CO_2	C=O
Water	H_2O	O-H
Methane	CH_4	C-H

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Further Reading

Explore

Watch this short video to hear more about bio-spectroscopy and its applications within research

Independent research

Professor Frank Martin - One Minute Research

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100 Black Prince Road
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