Spectroscopy

Teacher Notes
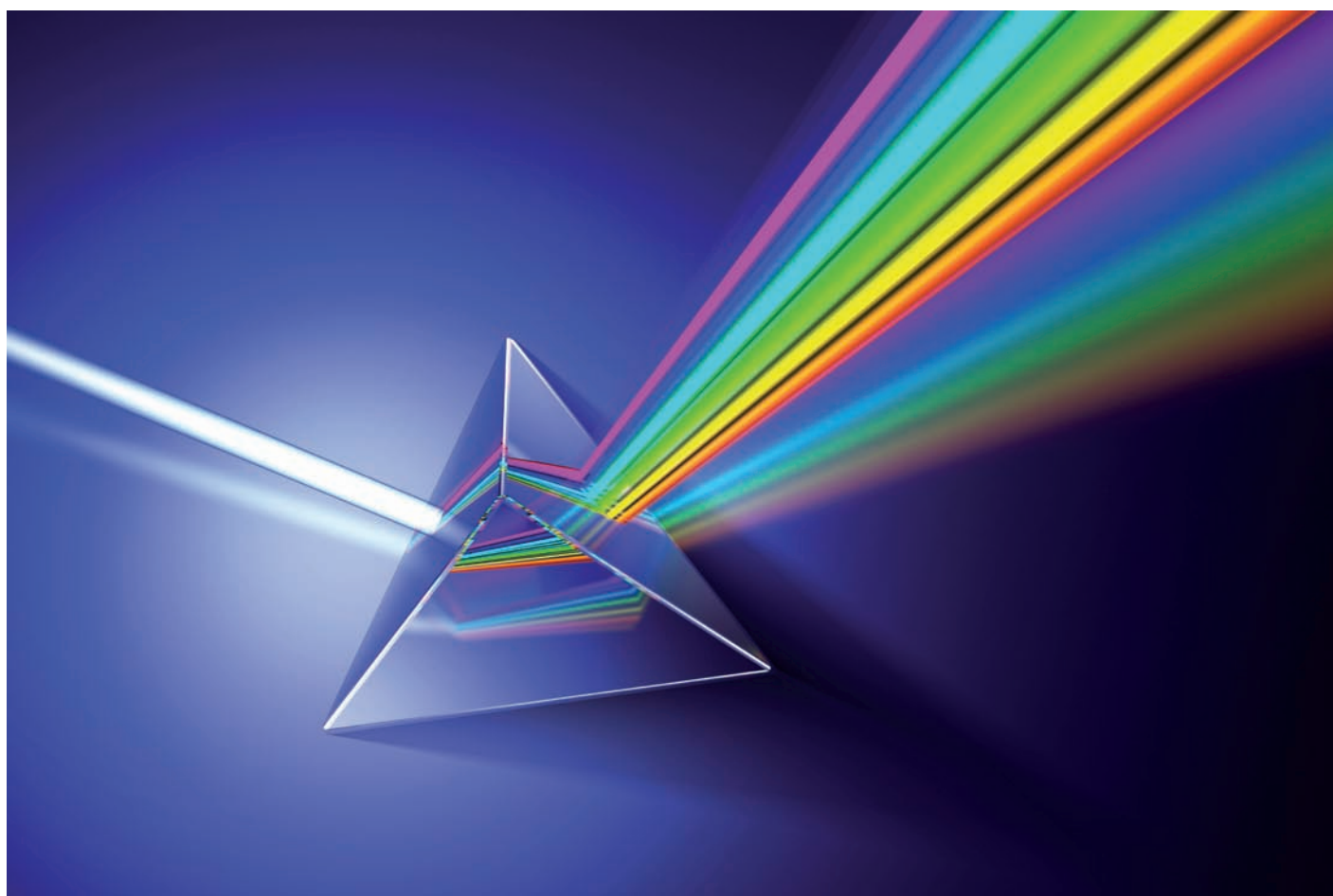
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Spectroscopy

Why focus on G&T and higher achievers?

Within the education system every child has the right to develop their learning so as to maximise their potential.

These exercises are designed to give students enthuse and enrich activities that although related to the curriculum are in fact taking the learning experience to the next level whilst also showing chemistry in a familiar context. This has been found to be a successful model for not only improving learning but also for raising levels of motivation. Higher achieving students can find the restraints of the standard curriculum to be demotivating leading to underachievement.

The different activities are designed to improve a number of skills including practical work/dexterity, thinking/analysis skills, literacy, research activities, use of models and teamwork. Students should also gain confidence through the activities and improve the ability to express themselves.

Some of the activities would appear to be complex for KS4, however at this stage in their learning high achieving students are open to new concepts and are ready to explore issues without pre-conceptions. They are keen to link ideas and develop concepts and understanding. It can prove to be an uplifting experience.

Introduction

Spectroscopy is an invaluable tool in both the qualitative and quantitative analysis of substances. In this set of activities the focus is on colourimetry, UV/Visible spectrometry and Fourier Transform Infrared (FTIR) spectrometry.

Students often have an awareness, although laden with misconceptions, of the use of spectroscopy from television programmes and cinema. This set of activities puts it in a true context through examining a familiar substance, namely aspirin

The programme is designed to develop students understanding of these topics from basic concepts to higher level thinking. It also aims to show that understanding how aspects of chemistry link together gives fuller understanding of the chemical processes as a whole. Working through the activities will also develop thinking and research skills.

Topic	Type of activity	Summary	Timing (mins)	KS3	KS4	KS5	Page
Spectrophotometric determination of concentration by colourimetry	Practical	This activity introduces colourimetry as a quantitative analysis tool linked to the Beer-Lambert Law.	45		√	√	15
Body in the Lab: Aspirin Overdose	Practical	As a development of the previous activity the analysis of aspirin. This is achieved by an indirect method forming a coloured complex with the Fe^{3+} ion.	60+		√	√	17

Body in the Lab: Structure determination	Practical/ Demonstration/ Paper exercise	Infrared spectroscopy applied to the analysis of aspirin. The activity explores functionality and structural determination.	60		√	√	28

These activities are designed for years 11 to 13. These are the only students who will have sufficient background knowledge in order to access the learning points.

The first activity is an introduction into spectroscopy using colourimeters that are common in many schools. It uses the Beer-Lambert relationship that states that absorbance is directly proportional to concentration in order to undertake a quantitative analysis of potassium manganate (VII) solutions. Students need to collect data and present this in graphical form in order to analyse the concentration of a solution of unknown concentration. It is important to remember that this only works for dilute solutions.

The body in the lab activity, again using the Beer-Lambert Law, investigates whether someone has died of an overdose of aspirin or not. It utilizes the fact that transition metal complexes are coloured. The aspirin is reacted with Fe^{3+} ions which produce a violet complex that is then analysed by UV/visible spectrometry, although a colourimeter can be used.

This analysis is continued by interpreting the FTIR spectra of different structures related to and including aspirin. Functional group recognition is practiced and linked to structure by adding in mass spectra into the equation.

Aims and objectives
The aims and objectives of these activities are:

- Developing questioning skills through problem solving.
- Exploring the use of models to expand understanding
- Develop practical skills and dexterity.
- Promote independent learning.
- Chemistry topics:
  o Beer-Lambert Law
  o Colourimetry
  o UV/Visible spectrometry
  o FT Infrared spectrometry
  o Functional groups
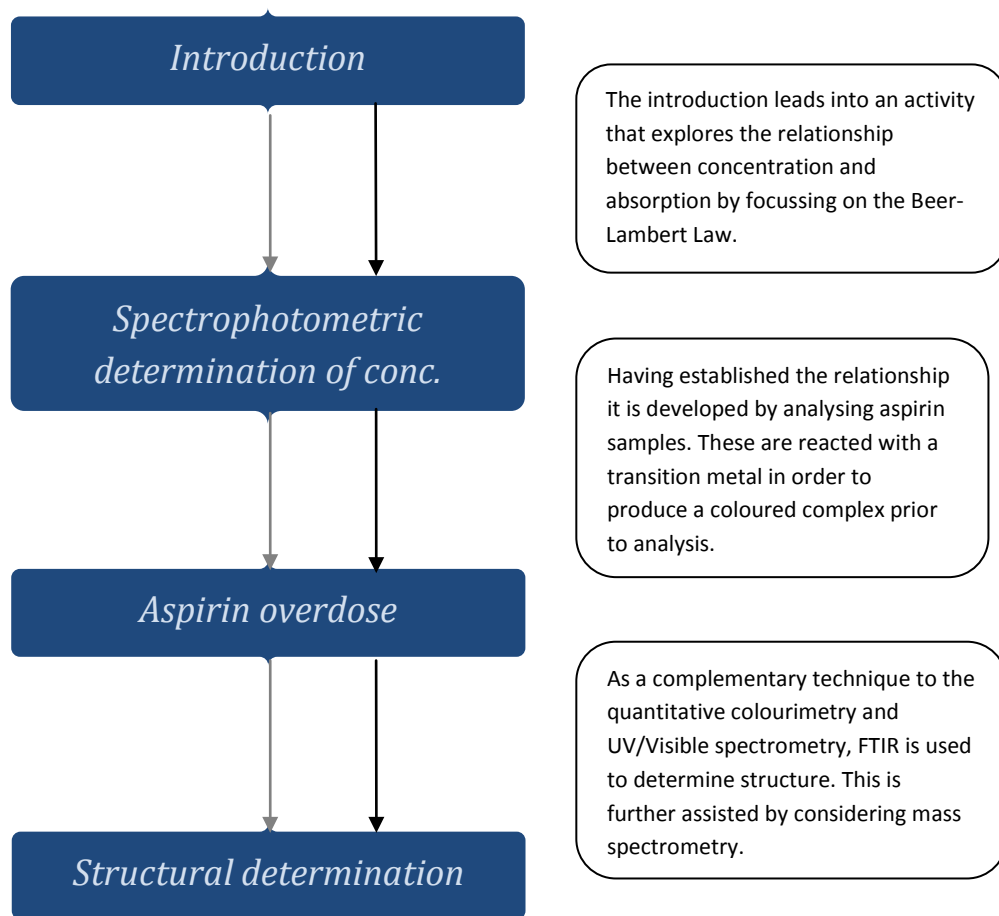 o Transition metal complexes

These exercises can be used with key stages 4 (year 11), and 5 as indicated on the *Possible Routes*.

These activities have proved very successful with key stage 4 year 11 students who have followed the prescribed pathway and have been stimulated into further independent learning.
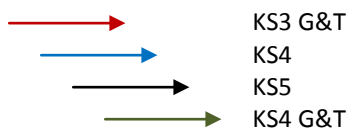
As well as developing key concepts and understanding, these exercises provide a reinforcement of a wide range of topics from the A level syllabus.

At all levels there is promotion of questioning skills, independent learning and research skills.

Possible routes

Introduction

> The introduction leads into an activity that explores the relationship between concentration and absorption by focussing on the Beer-Lambert Law.

Spectrophotometric determination of conc.

> Having established the relationship it is developed by analysing aspirin samples. These are reacted with a transition metal in order to produce a coloured complex prior to analysis.

Aspirin overdose

> As a complementary technique to the quantitative colourimetry and UV/Visible spectrometry, FTIR is used to determine structure. This is further assisted by considering mass spectrometry.

Structural determination

Key

→ (red)	KS3 G&T
→ (blue)	KS4
→ (black)	KS5
→ (green)	KS4 G&T

Introduction to Spectroscopy

What is spectroscopy?

One of the frustrations of being a chemist is the fact that no matter how hard you stare at your test tube or round-bottomed flask you can't actually see the individual molecules you have made! Even though your product looks the right colour and seems to give sensible results when you carry out chemical tests, can you be really sure of its precise structure?

Fortunately, help is at hand. Although you might not be able to 'see' molecules, they do respond when light energy hits them, and if you can observe that response, then maybe you can get some information about that molecule. This is where spectroscopy comes in.

Spectroscopy is the study of the way light (electromagnetic radiation) and matter interact. There are a number of different types of spectroscopic techniques and the basic principle shared by all is to shine a beam of a particular electromagnetic radiation onto a sample and observe how it responds to such a stimulus; allowing scientists to obtain information about the structure and properties of matter.

What is light?

Light carries energy in the form of tiny particles known as photons. Each photon has a discrete amount of energy, called a quantum. Light has wave properties with characteristic wavelengths and frequency (see the diagram below). The energy of the photons is related to the frequency (v) and wavelength (λ) of the light through the two equations:

$E = h v$ and $v = c / \lambda$

(where h is Planck's constant and c is the speed of light).

Therefore, high energy radiation (light) will have high frequencies and short wavelengths. The range of wavelengths and frequencies in light is known as the electromagnetic spectrum. This spectrum is divided into various regions extending from very short wavelength, high energy radiation (including gamma rays and X-rays) to very long wavelength, low energy radiation (including microwaves and broadcast radio waves).

The visible region (white light) only makes up a small part of the electromagnetic spectrum considered to be 380-770 nm. [Note that a nanometre is 10^{-9} metres].
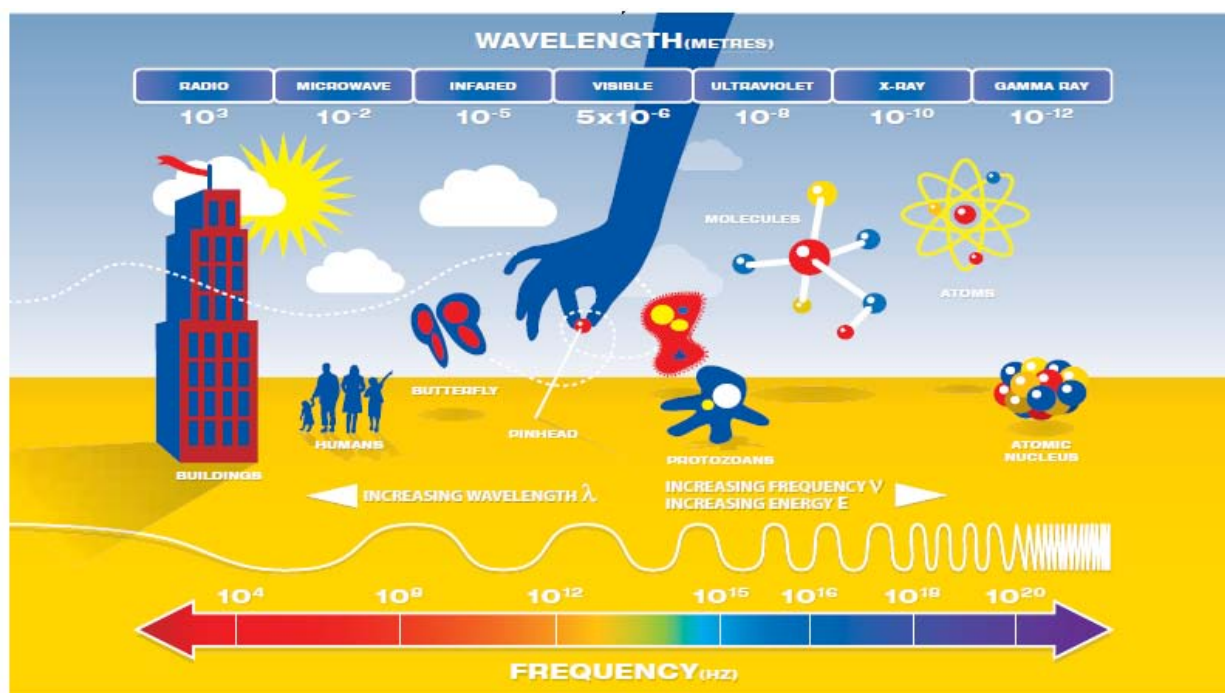
When matter absorbs electromagnetic radiation the change which occurs depends on the type of radiation, and therefore the amount of energy, being absorbed. Absorption of energy causes an electron or molecule to go from an initial energy state (ground state) to a high energy state (excited state) which could take the form of the increased rotation, vibration or electronic excitation. By studying this change in energy state scientists are able to learn more about the physical and chemical properties of the molecules.

- Radio waves can cause nuclei in some atoms to change magnetic orientation and this forms the basis of a technique called nuclear magnetic resonance (NMR) spectroscopy.
- Molecular rotations are excited by microwaves.
- Electrons are promoted to higher orbitals by ultraviolet or visible light.
- Vibrations of bonds are excited by infrared radiation.

The energy states are said to be quantised because a photon of precise energy and frequency (or wavelength) must be absorbed to excite an electron or molecule from the ground state to a particular excited state.

Since molecules have a unique set of energy states that depend on their structure, **IR, UV-visible** and **NMR** spectroscopy will provide valuable information about the structure of the molecule.

To 'see' a molecule we need to use light having a wavelength smaller than the molecule itself (approximately 10^{-10} m). Such radiation is found in the X-ray region of the electromagnetic spectrum and is used in the field of **X-ray crystallography**. This technique yields very detailed three-dimensional pictures of molecular structures – the only drawback being that it requires high quality crystals of the compound being studied. Although other spectroscopic techniques do not yield a three-dimensional picture of a molecule they do provide information about its characteristic features and are therefore used routinely in structural analysis.

Mass spectrometry is another useful technique used by chemists to help them determine the structure of molecules. Although sometimes referred to as mass spectro*scopy* it is, by definition, not a spectroscopic technique as it does not make use of electromagnetic radiation. Instead the molecules are ionised using

high energy electrons and these molecular ions subsequently undergo fragmentation. The resulting mass spectrum contains the mass of the molecule and its fragments which allows chemists to piece together its structure. In all spectroscopic techniques only very small quantities (milligrams or less) of sample are required, however, in mass spectrometry the sample is destroyed in the fragmentation process whereas the sample can be recovered after using IR, UV-visible and NMR spectroscopy.

| TECHNIQUE	RADIATION		WHAT CAN IT SEE?
Nuclear Magnetic Resonance (NMR) spectroscopy	Radio waves (10^3 m)	Electrons flipping magnetic spin 10^{-3} m	How neighbouring atoms of certain nuclei (e.g. ^1H, ^{13}C, ^{19}F, ^{31}P) in a molecule are connected together, as well as how many atoms of these type are present in different locations in the molecule.
Infra-red spectroscopy	Infra-red (10^5 m)	10^{-5} m NOTE Molecule vibrations	The functional groups which are present in a molecule.
UV-visible spectroscopy	Ultra-violet (10^8 m)	10^{-8} m NOTE Electrons promoted to higher energy state	Conjugated systems (i.e. alternating single and double bonds) in organic molecules as well as the metal-ligand interactions in transition metal complexes.
X-ray crystallography	X-rays (10^{10} m)	10^{-10} m x-ray	How all the atoms in a molecule are connected in a three-dimensional arrangement.
Mass spectrometry	Non-spectroscopic technique	Molecules fragment	The mass to charge ratio of the molecular ion (i.e. the molecular weight) and the fragmentation pattern which may be related to the structure of the molecular ion.

UV/Visible Spectroscopy

Absorption of ultraviolet and visible radiation

Absorption of visible and ultraviolet (UV) radiation is associated with **excitation of electrons**, in both atoms and molecules, from lower to higher energy levels. Since the energy levels of matter are quantized, only light with the precise amount of energy can cause transitions from one level to another will be absorbed. The possible electronic transitions that light might cause are:
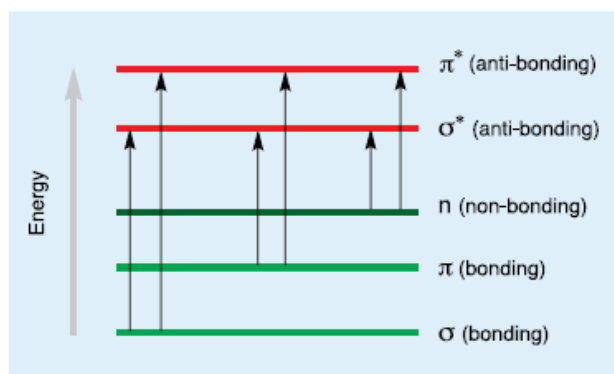
In each possible case, an electron is excited from a full (low energy, ground state) orbital into an empty (higher energy, excited state) anti-bonding orbital. Each wavelength of light has a particular energy associated with it. If that particular amount of energy is just right for making one of these electronic transitions, then that wavelength will be absorbed. The larger the gap between the energy levels, the greater the energy required to promote the electron to the higher energy level; resulting in light of higher frequency, and therefore shorter wavelength, being absorbed.

All molecules will undergo electronic excitation following absorption of light, but for most molecules very high energy radiation (in the vacuum ultraviolet, <200 nm) is required. Consequently, absorption of light in the UV-visible region will only result in the following transitions:
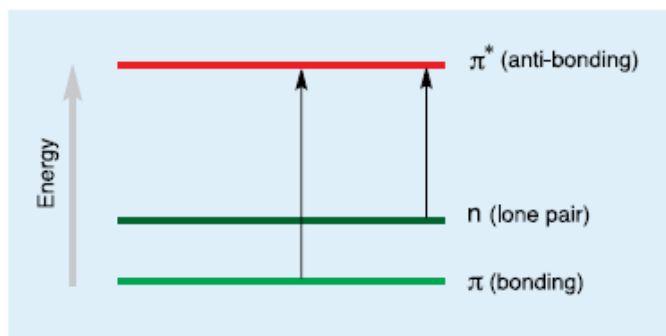
Therefore in order to absorb light in the region from 200 - 800 nm (where spectra are measured), the molecule must contain either C bonds or atoms with non-bonding orbitals. A non-bonding orbital is a lone pair on, say, oxygen, nitrogen or a halogen.

π bonds are formed by sideways overlap of the half-filled p orbitals on the two carbon atoms of a double bond. The two red shapes shown in the diagram below for ethene are part of the same π bonding orbital. Both of the electrons are found in the resulting π bonding orbital in the ground state.


sideways overlap of
half-filled p orbitals
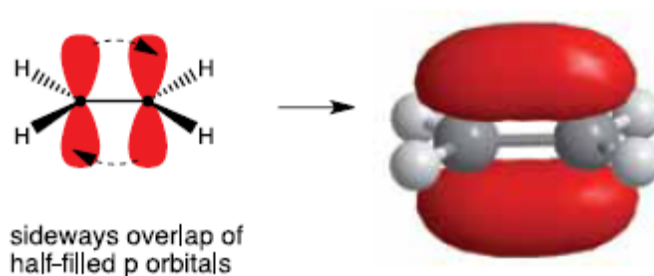
Molecules that contain **conjugated systems**, i.e. **alternating single and double bonds**, will have their electrons delocalised due to overlap of the p orbitals in the double bonds. This is illustrated below for buta-1,3-diene.
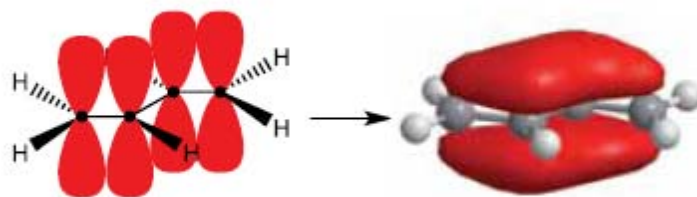
Benzene is a well-known example of a conjugated system. The Kekulé structure of benzene consists of alternating single double bonds and these give rise to the delocalised π system above and below the plane of the carbon – carbon single bonds.
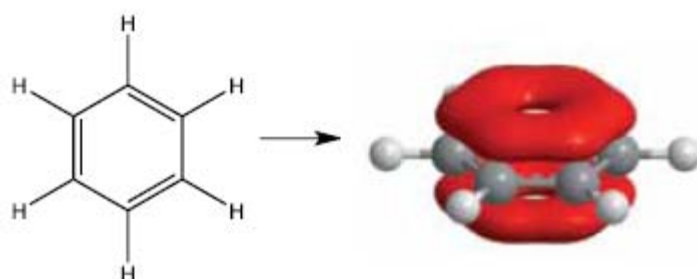
As the amount of delocalisation in the molecule increases the energy gap between the π bonding orbitals and π anti-bonding orbitals gets smaller and therefore light of lower energy, and longer wavelength, is absorbed.
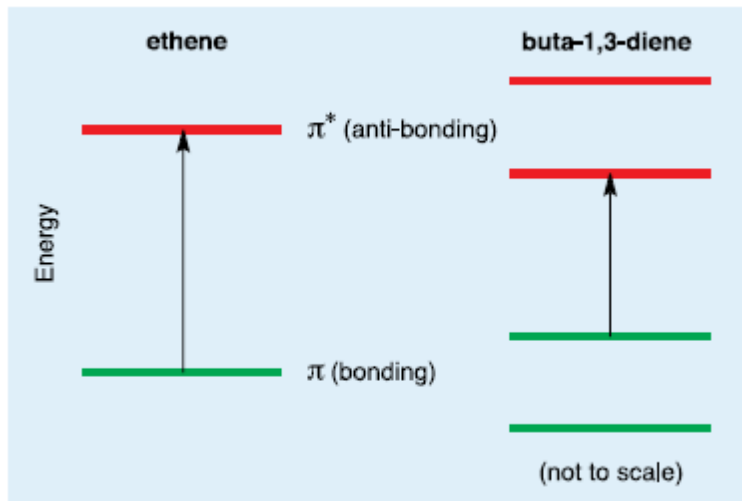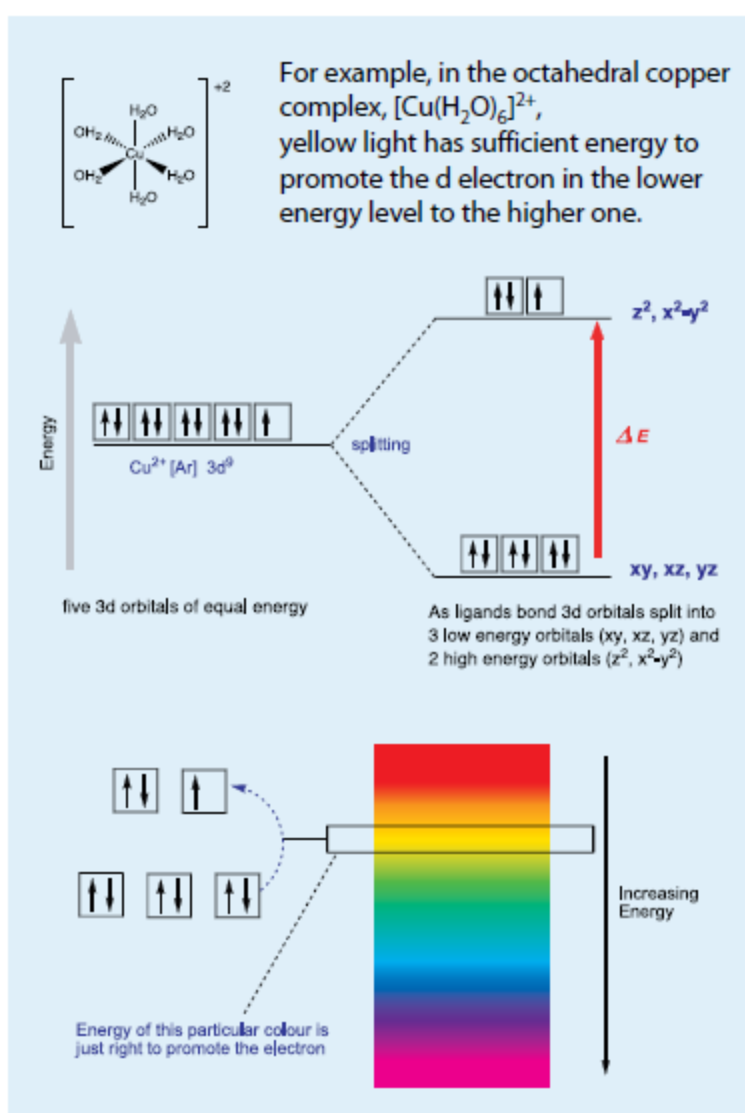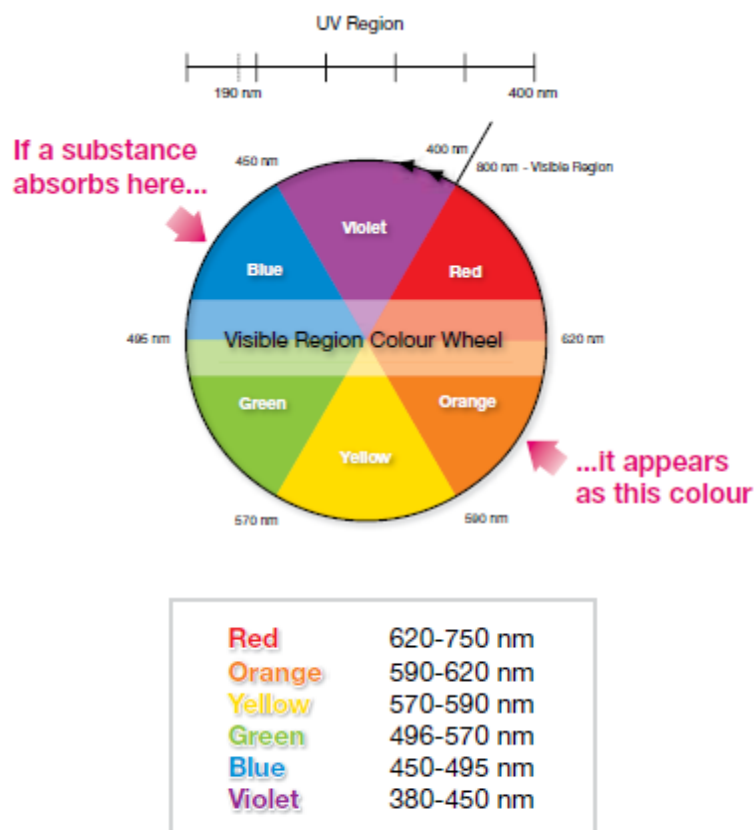

Although buta-1,3-diene absorbs light of a longer wavelength than ethene it is still absorbing in the UV region and hence both compounds are colourless. However, if the delocalisation is extended further the wavelength absorbed will eventually be long enough to be in the visible region of the spectrum, resulting in a highly coloured compound. A good example of this is the orange plant pigment, beta-carotene – which has 11 carbon-carbon double bonds conjugated together.
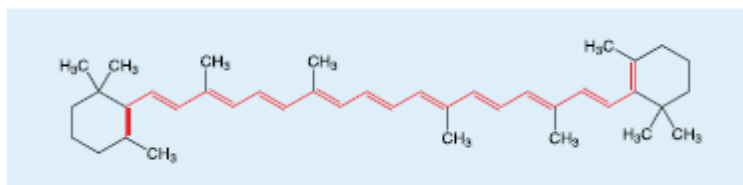
Beta-carotene absorbs throughout the UV region but particularly strongly in the visible region between 400 and 500 nm with a peak at 470 nm. Groups in a molecule which consist of alternating single and double bonds (conjugation) and absorb visible light are known as **chromophores**.

Transition metal complexes are also highly coloured, which is due to the splitting of the d orbitals when the ligands approach and bond to the central metal ion. Some of the d orbitals gain energy and some lose energy. The amount of splitting depends on the central metal ion and ligands. The difference in energy between the new levels affects how much energy will be absorbed when an electron is promoted to a higher level. The amount of energy will govern the colour of light which will be absorbed.

For example, in the octahedral copper complex, $[Cu(H_2O)_6]^{2+}$, yellow light has sufficient energy to promote the d electron in the lower energy level to the higher one.

five 3d orbitals of equal energy

Cu^{2+} [Ar] $3d^9$

splitting

ΔE

z^2, x^2-y^2

xy, xz, yz

As ligands bond 3d orbitals split into 3 low energy orbitals (xy, xz, yz) and 2 high energy orbitals (z^2, x^2-y^2)

Increasing Energy

Energy of this particular colour is just right to promote the electron

| Red | 620-750 nm |
Orange	590-620 nm
Yellow	570-590 nm
Green	496-570 nm
Blue	450-495 nm
Violet	380-450 nm

It is possible to predict which wavelengths are likely to be absorbed by a coloured substance. When white light passes through or is reflected by a coloured substance, a characteristic portion of the mixed wavelengths is absorbed. The remaining light will then assume the complementary colour to the wavelength(s) absorbed. This relationship is demonstrated by the colour wheel shown above. Complementary colours are diametrically opposite each other.

UV/Visible Spectrometer
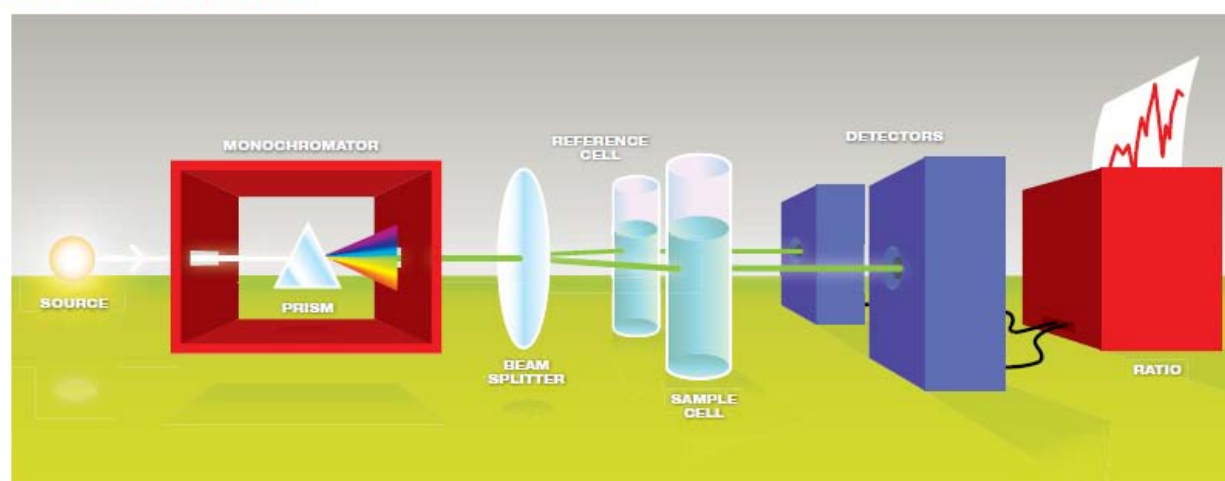
UV-visible spectrometers can be used to measure the absorbance of ultra violet or visible light by a sample, either at a single wavelength or perform a scan over a range in the spectrum. The UV region ranges from 190 to 400 nm and the visible region from 400 to 800 nm.

The technique can be used both quantitatively and qualitatively. A schematic diagram of a UV-visible spectrometer is shown above. The light source (a combination of tungsten/halogen and deuterium lamps) provides the visible and near ultraviolet radiation covering the 200 – 800 nm. The output from the light source is focused onto the diffraction grating which splits the incoming light into its component colours of different wavelengths, like a prism (shown below) but more efficiently.
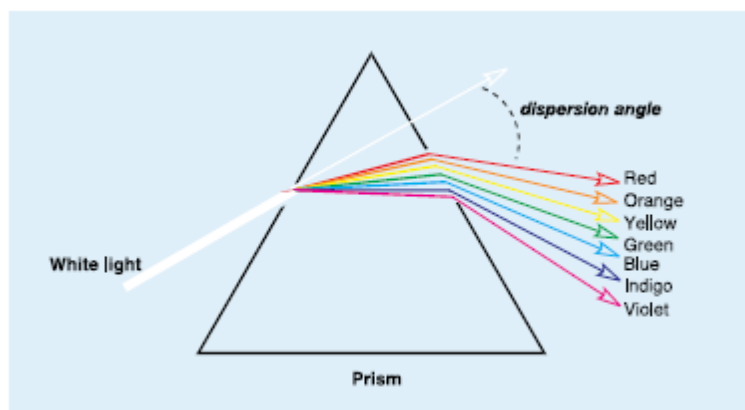
For liquids the sample is held in an optically flat, transparent container called a cell or cuvette. The reference cell or cuvette contains the solvent in which the sample is dissolved and this is commonly referred to as the blank. For each wavelength the intensity of light passing through both a reference cell (I_o) and the sample cell (I) is measured. If I is less than I_o, then the sample has absorbed some of the light.

The absorbance (A) of the sample is related to I and Io according to the following equation:

$$A = \log_{10} \frac{I_o}{I}$$

The detector converts the incoming light into a current, the higher the current the greater the intensity. The chart recorder usually plots the absorbance against wavelength (nm) in the UV and visible section of the electromagnetic spectrum *(Note: absorbance does not have any units).*
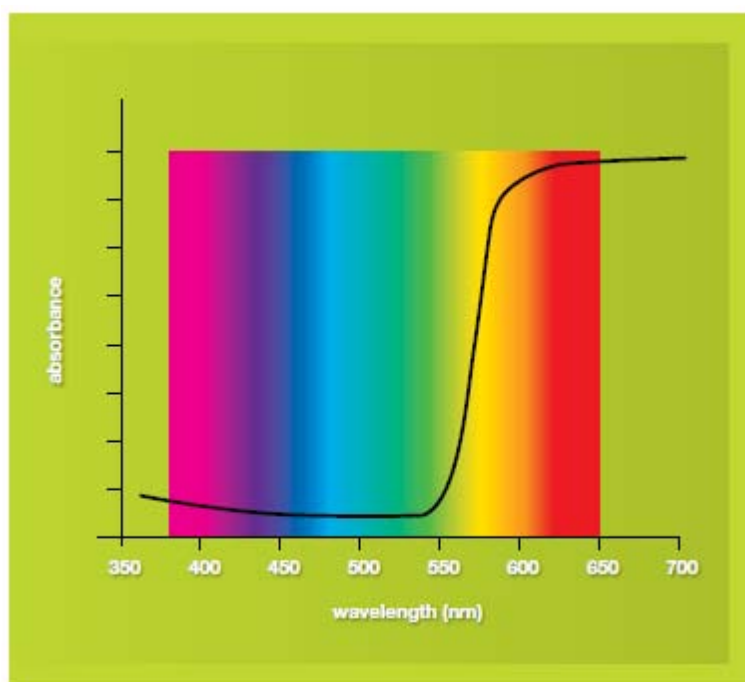
UV-Visible Spectrum

The diagram below shows a simple UV-visible absorption spectrum for buta-1,3-diene. Absorbance (on the vertical axis) is just a measure of the amount of light absorbed. One can readily see what wavelengths of light are absorbed (peaks), and what wavelengths of light are transmitted (troughs). The higher the value, the more of a particular wavelength is being absorbed.

Maximum absorption at this wavelength

λ max=217nm
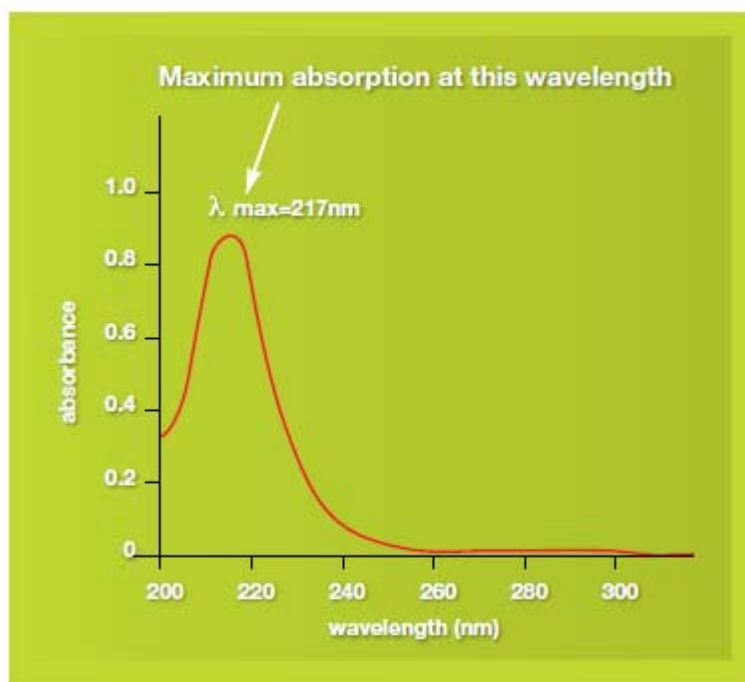
The absorption peak at a value of 217 nm, is in the ultra-violet region, and so there would be no visible sign of any light being absorbed making buta-1,3-diene colourless. The wavelength that corresponds to the highest absorption is usually referred to as "lambda-max" (λ_{max}).

The spectrum for the blue copper complex shows that the complementary yellow light is absorbed.

The Beer-Lambert Law

According to the Beer-Lambert Law the absorbance is proportional to the concentration of the substance in solution and as a result UV-visible spectroscopy can also be used to measure the concentration of a sample.

The Beer-Lambert Law can be expressed in the form of the following equation:

$$A = \varepsilon cl$$

Where

A = absorbance
l = optical path length, i.e. dimension of the cell or cuvette (cm)
c = concentration of solution (mol dm^{-3})
ε = molar extinction, which is constant for a particular substance at a particular wavelength (dm^3 mol^{-1} cm^{-1})

If the absorbance of a series of sample solutions **of known concentrations are measured and plotted against their corresponding concentrations, the plot of absorbance versus concentration should be linear if the Beer-Lambert Law is obeyed. This graph is known as a calibration graph.**

A calibration graph can be used to determine the concentration of unknown sample solution by measuring its absorbance, as illustrated below.
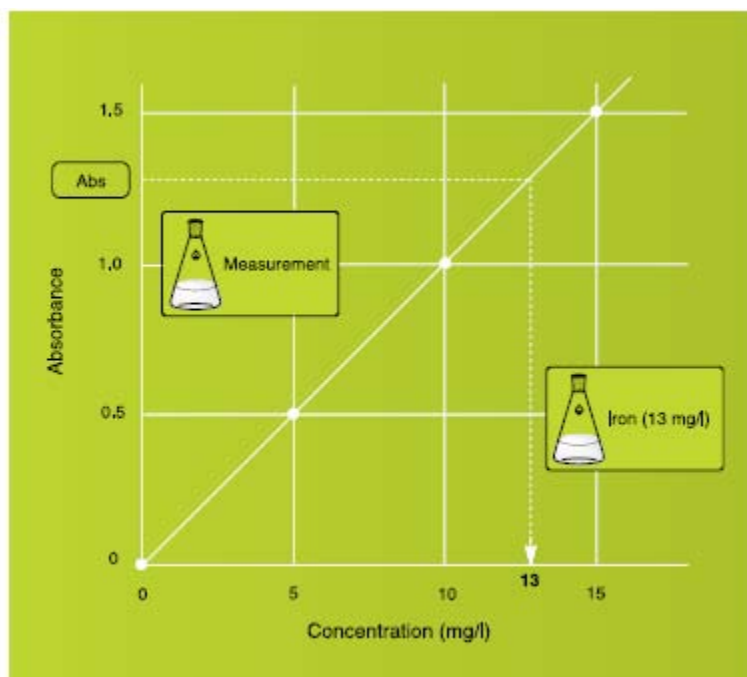
Since the absorbance for dilute solutions is directly proportional to concentration another very useful application for UV-visible spectroscopy is studying reaction kinetics. The rate of change in concentration of reactants or products can be determined by measuring the increase or decrease of absorbance of coloured solutions with time. Plotting absorbance against time one can determine the orders with respect to the reactants and hence the rate equation from which a mechanism for the reaction can be proposed.

Modern Applications of UV Spectroscopy

UV-visible spectroscopy is a technique that readily allows one to determine the concentrations of substances and therefore enables scientists to study the rates of reactions, and determine rate equations for reactions, from which a mechanism can be proposed. As such UV spectroscopy is used extensively in teaching, research and analytical laboratories for the quantitative analysis of all molecules that absorb ultraviolet and visible electromagnetic radiation.

Other applications include the following:

- In clinical chemistry UV-visible spectroscopy is used extensively in the study of enzyme kinetics. Enzymes cannot be studied directly but their activity can be studied by analysing the speed of the reactions which they catalyse. Reagents or labels can also be attached to molecules to permit indirect detection and measurement of enzyme activity. The widest use in the field of clinical diagnostics is as an indicator of tissue damage. When cells are damaged by disease, enzymes leak into the bloodstream and the amount present indicates the severity of the tissue damage. The relative proportions of different enzymes can be used to diagnose disease, say of the liver, pancreas or other organs which otherwise exhibit similar symptoms.

- UV-visible spectroscopy is used for dissolution testing of tablets and products in the pharmaceutical industry. Dissolution is a characterisation test commonly used by the pharmaceutical industry to guide formulation design and control product quality. It is also the only test that measures the rate of *in-vitro* drug release as a function of time, which can reflect either reproducibility of the product manufacturing process or, in limited cases, *in-vivo* drug release.

- In the biochemical and genetic fields UV-visible spectroscopy is used in the quantification of DNA and protein/enzyme activity as well as the thermal denaturation of DNA.

- In the dye, ink and paint industries UV-visible spectroscopy is used in the quality control in the development and production of dyeing reagents, inks and paints and the analysis of intermediate dyeing reagents.

- In environmental and agricultural fields the quantification of organic materials and heavy metals in fresh water can be carried out using UV-visible spectroscopy.

The first activity introduces the idea of spectrometry through colourimetry. It also looks at spectrometry as a quantitative tool through the link with the Beer-Lambert Law. The apparatus necessary is available in many schools.

Activity 1: Spectrophotometric measurement of concentration using colourimeters

Teacher notes

This experiment uses colourimeters and is reliant upon Beer-Lamberts law whereby there is a proportional, straight line relationship between absorbance and concentration (for low concentration solutions). In this experiment you will be using solutions of potassium manganate VII.

This experiment follows the following steps:
1. Prepare the solutions
2. The stock solution provided contains 120 mg dm^{-3}. You are going to use this as your highest concentration. You will also need to dilute this with distilled water in order to complete the following table.

Conc. mg dm^{-3}	0	20	40	60	80	100	120
Absorbance A							

3. Finding the wavelength of maximum absorbance
4. Using the 60 mg dm^{-3} solution (a mid range solution) change the filter on the colourimeter to find out which wavelength gives the highest reading for A.

Measuring absorbance

At the wavelength from 2 record the absorbance for your blank (the solvent) by placing a cuvette containing the solvent into the colourimeter and pressing R. Replace the blank cuvette with all of your solutions, pressing T on each occasion and recording the reading.

Plotting a calibration graph

Using the same wavelength from 3 record the absorbance for all of your solutions. Plot this information on the graph paper. Concentration on X axis. Plot a line of best fit through the origin.

Concentration of the unknown solution.

Using the same wavelength measure the absorbance of the unknown solution. Now use your calibration graph to determine the unknown concentration.
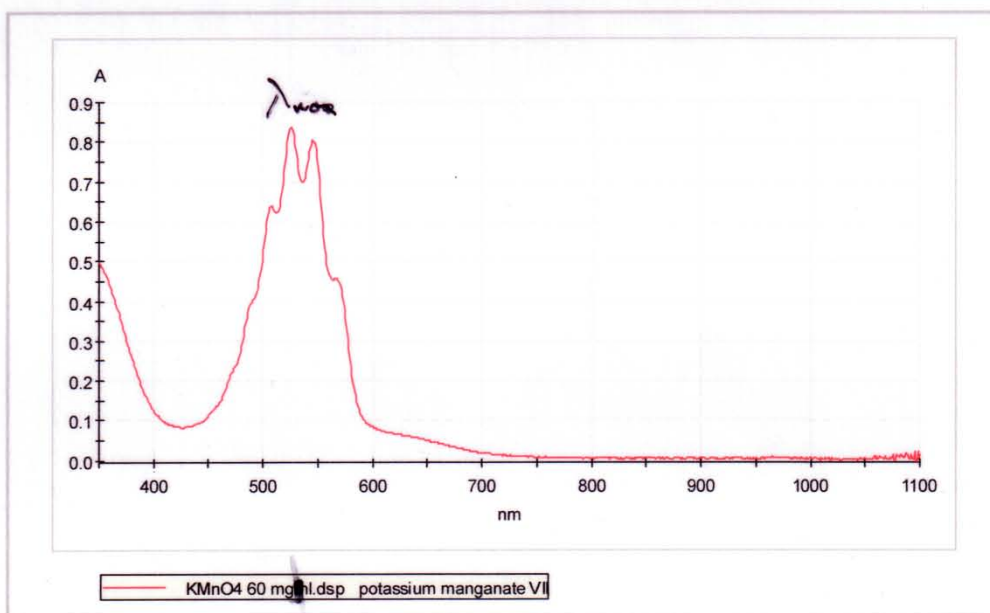
Concentration of unknown = _____ mg dm^{-3}

UV/Vis   Potassium manganate VII   585nm
0.831A

Spectrum: KMnO4 60 mgml.dsp
Description: potassium manganate VII
Operator: DOMAIN\andrew thompson (Andrew Thompson)
Created: 08/06/2011 10:09:03
Spectrophotometer: GENESYS 10S UV-Vis
Serial number: 2L9N144004
Firmware: 4.002
Baseline: 08/06/2011 09:42:54

KMnO4 60 mgml.dsp   potassium manganate VII

Set filter   520nm/
             550nm

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Spectrophotometric measurement of concentration using colourimeters

Technician notes

Chemicals

    1 dm^3 KMnO$_4$ stock solution containing 120 mg dm^{-3}. (solid - **oxidising**, **harmful**, solution – **low hazard**) See CLEAPSS Hazcard 81

    De-ionised water

Apparatus

    4 burettes
    4 funnels
    4 burette/retort stands

The same principles of the last activity are further developed in the next experiment where the concentration of aspirin is measured by UV/Visible spectrometry, although colourimeters may be used, by preparing a coloured complex with Fe^{3+} ions.

Activity 2: Body in a Lab: Aspirin Overdose

Teacher notes

Introduction

A body has been found in the Lab! The deceased, Mr Blue, was known to be taking aspirin and a sample of his blood plasma has been sent for analysis. Use UV spectroscopy to determine the concentration of aspirin in the body and ascertain if the amount present was enough to be the cause of death.
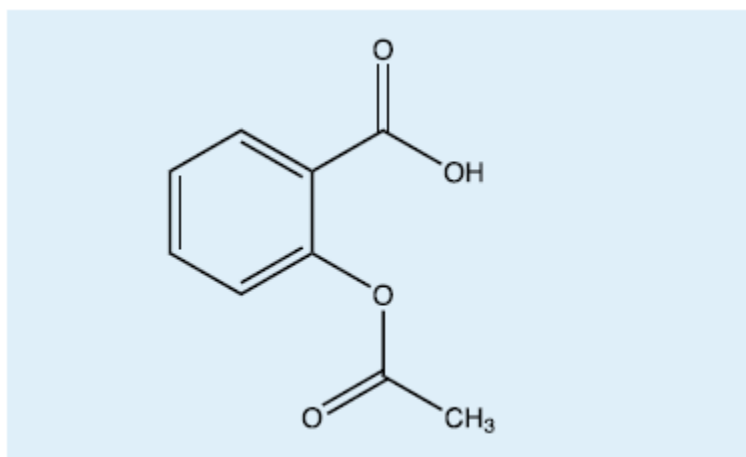
Analysis of Salicylate in Blood Plasma by UV-Visible Spectroscopy

Aspirin or acetyl salicylic acid is a widely available drug with many useful properties. It was one of the first drugs to be commonly available and it is still widely used with approximately 35,000 tonnes produced and sold each year, equating to approximately 100 billion aspirin tablets.
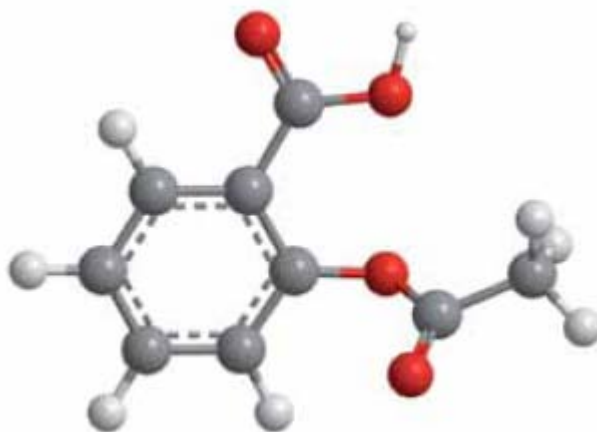
Aspirin is prepared by the acetylation of salicylic acid using acetic anhydride. Its many properties as a drug include its uses as an analgesic to reduce pain, anti-inflammatory to reduce inflammation, antipyretic to reduce temperature, and platelet aggregation inhibitor to thin the blood and stop clotting.

Therapeutic levels taken after a heart attack are typically 150 – 300 mg/L and for post by-pass operations 75 mg/L. The levels of salicylate present in blood plasma can be analysed using UV-visible spectroscopy to indicate if the subject has taken a therapeutic dose or an overdose (see following table).

Therapeutic	< 300 mg/L
Moderate Overdose	500 – 750 mg/L
Severe Overdose	>750 mg/L

Most adult deaths occur when the measured plasma level is greater than 700 mg/L. (Note: the maximum salicylate plasma levels usually occur approximately 4-6 hrs after ingestion).

Method

This method involves measuring the absorbance of the redviolet complex of ferric and salicylate ions at about 530 nm using a UV/Visible spectrometer. It is possible to use a colourimeter should a UV/Visible spectrometer is not available.

A 5% iron (III) chloride solution has been prepared for you (5g iron (III) chloride in 100 ml of de-ionised water).

1. Preparation of Salicylate Calibration Standards

A stock solution of 2000 mg/L salicylate in 250 ml of de-ionised water has been prepared for you by dissolving 580 mg sodium salicylate in a 250 ml volumetric flask.

Make up Standard Calibration Solutions (if this has not already been done for you)

In 100 ml standard volumetric flask dilute appropriate volumes of the stock solution to give 100, 200, 300, 400, and 500 mg/L salicylate calibration standards using the dilutions given below.

CONCENTRATION	DILUTION
100 mg/L	5 ml Stock Salicylate Solution in 100 ml De-ionised water
200 mg/L	10 ml Stock Salicylate Solution in 100 ml De-ionised water
300 mg/L	15 ml Stock Salicylate Solution in 100 ml De-ionised water
400 mg/L	20 ml Stock Salicylate Solution in 100 ml De-ionised water
500 mg/L	25 ml Stock Salicylate Solution in 100 ml De-ionised water

2. Prepare a Blank
In a test tube prepare a blank solution by taking 1 ml of de-ionised water and adding 4 ml of 5% iron (III) chloride solution.

3. Prepare Standards and Unknown Plasma Sample for UV/Vis Analysis
Prepare each of the standards and the unknown plasma sample by pipetting 1 ml into a separate test tubes and adding 4 ml of 5% iron (III) chloride solution to each (making sure each is carefully mixed).

4. Record the Absorbance
Transfer the calibration solutions, blank and unknown sample to separate cuvettes to record the absorbance. For each sample record the absorbance in the visible region between 400 – 600 nm. A peak should be observed at about 530 nm (see your demonstrator for instructions on using the UV/Visible spectrometer).

Analysis of results

1. Using the Beer-Lambert law plot the absorbance versus concentration calibration graph for the standards and using this find the unknown concentration of the salicylate present in the plasma.

2. Use this result to decide if the subject had taken a therapeutic or life threatening dose.
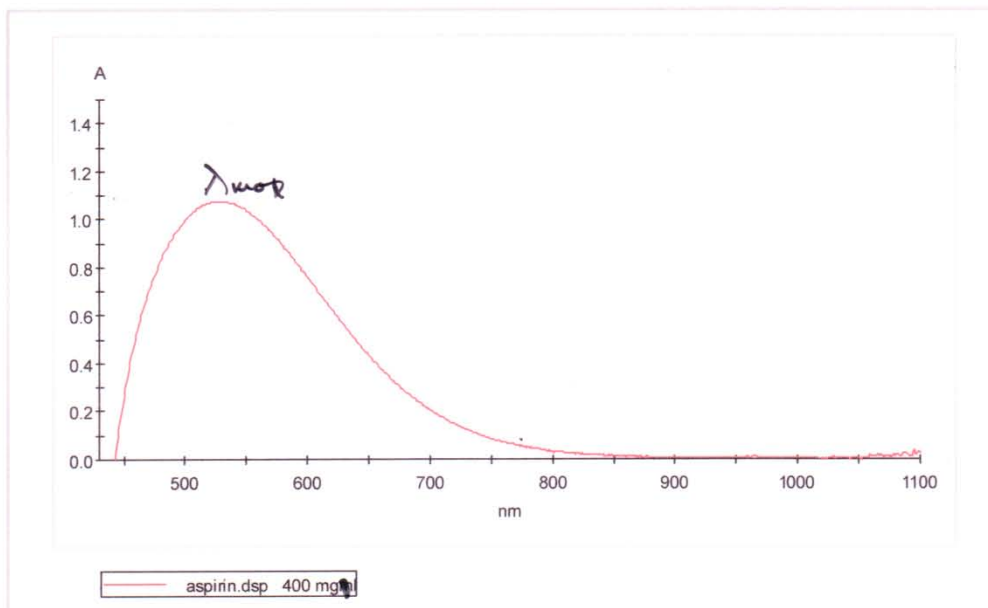
References

1. *Encyclopaedia of Analytical Science*, ed. Paul Worsfold, Alan Townshend, Colin Poole, Worsfold, Paul J. (2005), 543.003 ENC
2. *The Aspirin Foundation*
http://www.aspirin-foundation.com/what/index.htm
3. *Article by Professor A.N.P. van Heijst/ Dr A. van Dijk*
http://www.inchem.org/documents/pims/pharm/aspirin.htm
4. *Analysis of Analgesics*
http://faculty.mansfield.edu/bganong/biochemistry/aspirin.htm
5. *TLC of Analgesic Drugs*
http://www2.volstate.edu/msd/CHE/122/Labs/TLC.htm

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UV/VIS   ASPIRIN   528 nm
                                                1.075 A

Spectrum: aspirin.dsp
Description: 400 mgml
Operator: DOMAIN\andrew thompson (Andrew Thompson)
Created: 08/06/2011 11:32:45
Spectrophotometer: GENESYS 10S UV-Vis
Serial number: 2L9N144004
Firmware: 4.002
Baseline: 08/06/2011 11:31:40

λmax

aspirin.dsp   400 mgml

Set machine 528nm

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THE WOLFSON FOUNDATION

20

Body in a Lab: Aspirin Overdose

Technician notes

Chemicals

• De-ionised water

• 50 ml per group 5% iron (III) chloride solution (5g iron (III) chloride in 100 ml de-ionised water) (solid- **harmful**, solution – **irritant**) See CLEAPSS Hazcard 55C

• 2000 mg/L stock salicylate solution for preparing calibration standard solutions (580 mg sodium salicylate (solid- **harmful**; solution - **low hazard –** See CLEAPSS Hazcard 52) in 250 ml de-ionised water) give 100 ml per group if students to prepare standard solutions themselves.

• 1 ml per group unknown salicylate solution (12.5 ml stock salicylate in 100 ml de-ionised water)

• 1ml per group standard solutions

The following will have to be prepared in advance due to the shortage of time.

Concentration	Stock Salicylate Solution in 100 ml De-ionised water
100 mg/L	5 ml
200 mg/L	10 ml
300 mg/L	15 ml
400 mg/L	20 ml
500 mg/L	25 ml

Apparatus

Items per group

• 7 x test tubes and test tube rack
• 5 ml graduated pipette
• 1 ml graduated pipette
• Wash bottle
• Pipette filler
• Disposable pipettes for filling cuvettes

Infrared Spectroscopy

Infrared Spectroscopy (IR)

One of the first scientists to observe infrared radiation was William Herschel in the early 19th century. He noticed that when he attempted to record the temperature of each colour in visible light, the area just beyond red light gave a marked increase in temperature compared to the visible colours.

However it was only in the early 20th century that chemists started to take an interest in how infrared radiation interacted with matter and the first commercial infrared spectrometers were manufactured in the USA during the 1940s.

Interaction with matter

The bonds within molecules all vibrate at temperatures above absolute zero. There are several types of vibrations that cause absorptions in the infrared region. Probably the most simple to visualise are bending and stretching, examples of which are illustrated below using a molecule of water.

If the vibration of these bonds result in the change of the molecule's dipole moment then the molecule will absorb infrared energy at a frequency corresponding to the frequency of the bond's natural vibration. This absorption of energy resulting in an increase in the amplitude of the vibrations is known as **resonance**.

An IR spectrometer detects how the absorption of a sample varies with wavenumber, cm^{-1}, which is the reciprocal of the wavelength in cm (1/wavelength). The wavenumber is proportional to the energy or frequency of the vibration of the bonds in the molecule.

Symmetric stretch   Asymmetric stretch   Bend

Vibration in the x-axis   Vibration in the y-axis   Vibration in the z-axis
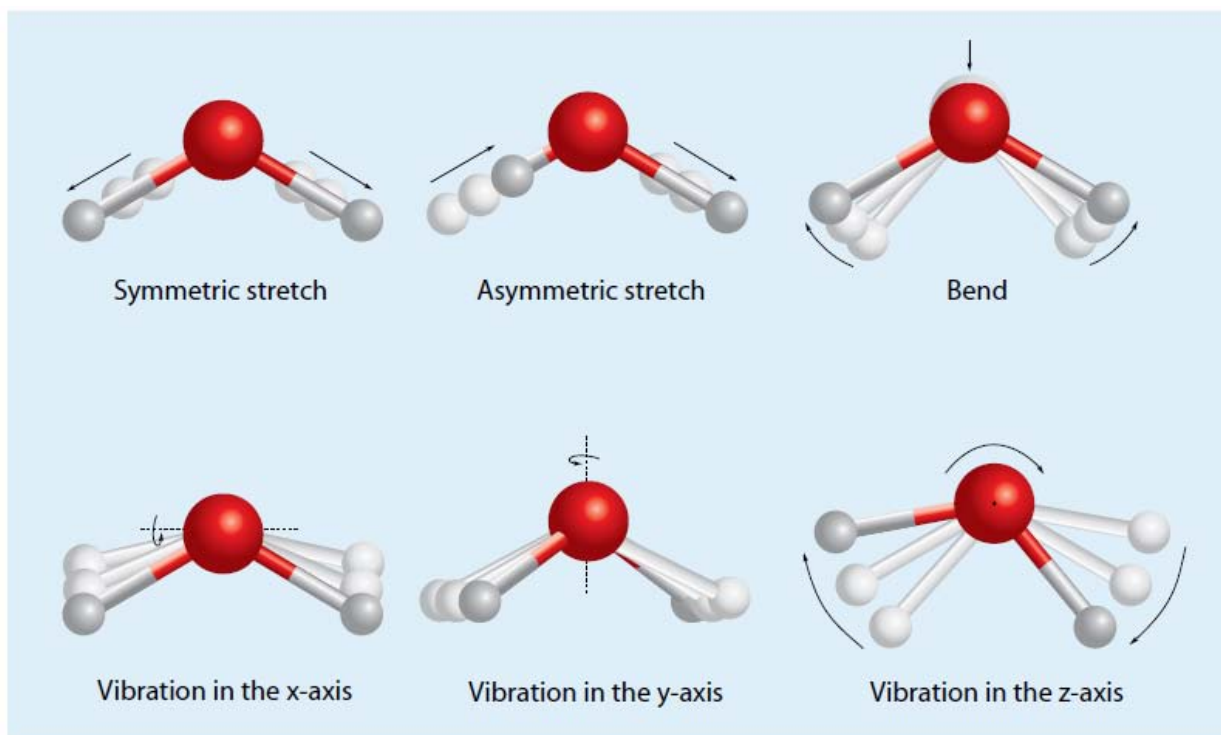
Carbon dioxide and IR

Carbon dioxide is probably the molecule most people associate with the absorption of infrared radiation, as this is a key feature of the greenhouse effect. If carbon dioxide was perfectly still it would not have a permanent dipole moment as its charge would be spread evenly across both sides of the molecule.

However the molecule is always vibrating and when it undergoes an asymmetric stretch, an uneven distribution of charge results. This gives the molecule a temporary dipole moment, enabling it to absorb infrared radiation. Hence even some molecules without a permanent uneven distribution of charge can absorb IR radiation.

(a) No permenant dipole moment when the molecule is stationary due to equal and opposite dipoles.
(b) As molecules undergo assymmetric stretch the permenant dipoles become uneven and temporary dipole moments are created.

Factors that affect vibrations

Type of Vibration

The energy absorbed when particular bonds vibrate depends on several factors. To get your head around this it is helpful to use an analogy; you can think of a bond as a spring between two atoms. Imagine trying to bend or stretch the spring. Generally it is easier to bend than stretch, so bending vibrations are of lower energy than stretching vibrations for the same bond. Therefore, absorptions due to bending tend to occur at lower wavenumbers than stretches.

Higher Energy

Stretch

Lower Energy
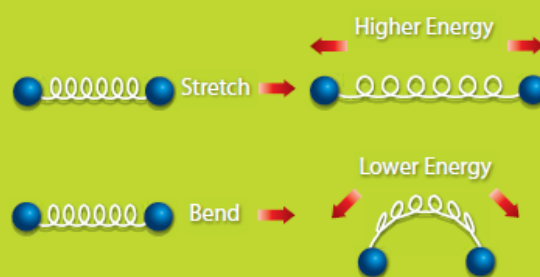
Bend

Hence a C–H Stretch in an alkane absorbs at 2600-2800 cm^{-1} but a C–H Bend in an alkane absorbs at 1365-1485 cm^{-1}

Strength of Bonds

You can think of a strong bond as a stiff spring. This will need more energy to make the 'spring' bond vibrate, so stronger bonds absorb at higher wavenumbers.

Single bond
weaker, so lower energy

Triple bond
stronger, so higher energy
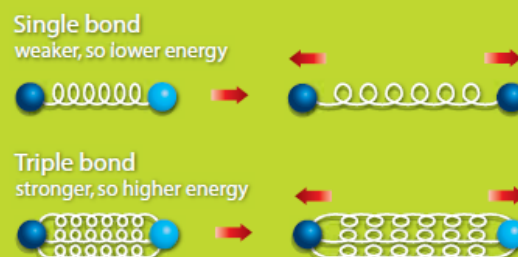
Hence C–N absorbs at roughly 1050 – 1650 cm^{-1} but a C≡N absorbs at roughly 2200 cm^{-1}

Mass of Atoms

Finally the atoms in the bond can be thought of as masses at the end of the spring. Heavy masses on a spring vibrate more slowly than lighter ones. Using this analogy we can imagine that heavier atoms vibrate at a lower frequency than lighter ones. Therefore you would expect a C-Br bond to absorb at a lower frequency than a C-Cl bond as bromine is heavier than chlorine.

Atom with less mass so faster vibration

Atom with greater mass so slower vibration
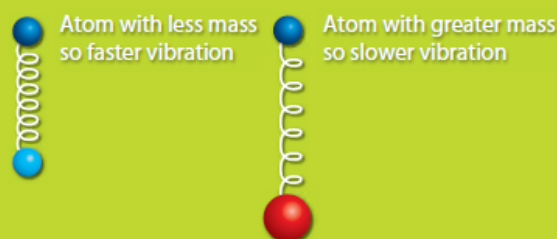
Hence C–Cl bond absorbs at about 600-800 cm^{-1} but a C–Br bond absorbs at about 500-600 cm^{-1}

Infrared Spectrometers

Infrared spectrometers work in a variety of ways but all of them pass infrared radiation across the full IR range through a sample. The sample can be a thin film of liquid between two plates, a solution held in special cells, a mull (or paste) between two plates or a solid mixed with potassium bromide and compressed into a disc. The cells or plates are made of sodium or potassium halides, as these do not absorb infrared radiation.

However the IR spectrometer 'in the suitcase' can use a technique called attenuated total reflectance (ATR), which allows IR spectra to be run on solid and liquid samples without any additional preparation.

Interpreting a spectrum

As molecules often contain a number of bonds, with many possible vibrations, an IR spectrum can show absorptions at many frequencies. This can be helpful as it results in each molecule's spectrum being unique. If the spectrum of a molecule has already been recorded on a database, any spectrum produced can be compared to that database to help identify the molecule. The spectrum can also be used to highlight specific bonds and hence functional groups within a molecule, to help determine its structure.

Look at this spectrum of ethanol, CH_3CH_2OH, to see which bonds are responsible for particular absorptions.

Although IR is not able to provide enough information to find the exact structure of a 'new' molecule, in conjunction with other spectroscopic tools, such as NMR and mass spectrometry, IR can help provide valuable information to help piece together the overall structure.

Ethanol
CH_2CH_3OH
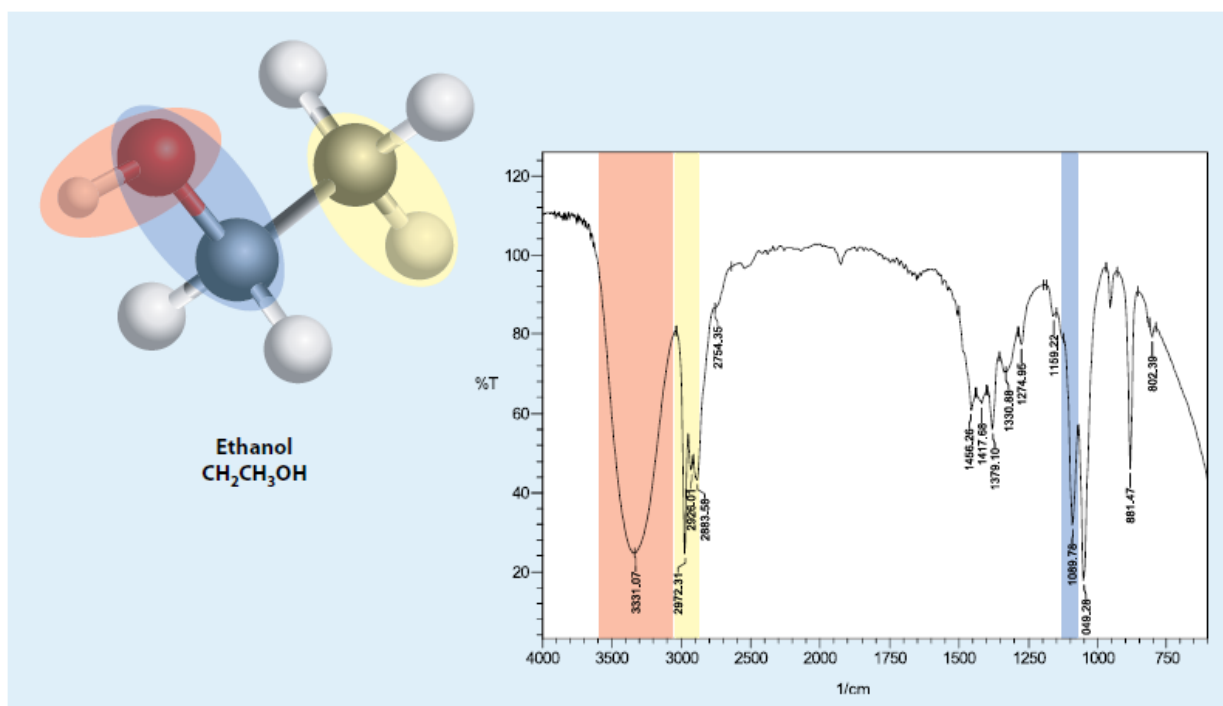
Uses of IR spectroscopy

Students and research chemists regularly use IR in structure determination and IR continues to have a wide range of applications in both research chemistry and wider society. For instance, IR is being used to help identify the structure of complex molecules in space and to help determine the time scale for the folding of complex protein structures into functioning biological molecules.

Many police forces across the world now routinely use IR, almost certainly without realising it. This is because many 'breathalysers' used to collect evidence to determine levels of alcohol in breath are IR

spectrometers that look specifically for absorptions at around 1060 cm^{-1}, which corresponds the vibration of the C-O bond in ethanol!

BASIC ORGANIC FUNCTIONAL GROUP REFERENCE

Structures

Methyl	Methylene			Alkene
CH₃—	—CH₂—			C=C
				Alkyne
				C≡C

Vinyl	Vinylidene	Cis	Trans
R₁ C=C (H, H)	R₁/R₂ C=C (H, H)	R₁ C=C R₂ (H, H)	R₁ C=C (H, H) R₂

Mono	Ortho	Meta	Para
R₁ (benzene)	R₁ R₂ (benzene)	R₁ R₂ (benzene)	R₁ R₂ (benzene)

Ether	Alcohol
C—O—C	—OH

Aldehyde	Ketone	Ester	Carboxylic Acid
O=C—H	O=C (C, C)	O=C—O—C	O=C—OH

Amide	Amine	Nitrile
O=C—N	C—N	C≡N

IR Reference Values (cm⁻¹)

Aliphatic — C—H

	C—H
Methyl	2960
Methylene	2930

Unsaturated — C=C

		C=C
Alkenes	3050	1640
Vinyl	910	1640
Vinylidene	890	1640
Cis	700	1640
Trans	965	1670
Alkynes (C≡C)	3200	2200

Aromatics

Mono	750	700
Ortho	750	-
Meta	782	700
Para	817	-

Oxygen Group — C—O, O—H

	C—O	O—H
Ether	1100	
Alcohol	1100	3100

Carbonyl Compounds — C=O

		C=O
Aldehyde	2700	1750 / 1730
Ketone		1700
Ester	1200	1740
Carboxylic Acid	3100	1720

Nitrogen Groups — N—H, C≡N

	N—H	C≡N
Amide	3200 (1640 C=O)	
Amine	3300	
Nitrile		2250

The aspirin theme is further developed in this FTIR activity. It is useful to see the instrument in operation, but if this is not possible video is available on the website indicated in the introduction. The remaining tasks can then become a paper exercise that develop the students interpretation of spectra leading to an understanding of functionality which when linked to mass spectrometry spectra can be used to determine structure.

Activity 3: Body in a Lab: Compound determination

Teacher notes

Introduction

If an FTIR spectrometer is not available there is sufficient information and number of spectra for this to become a paper exercise supported by the video and examples in the following website:

http://spectraschool.org

Background

A body has been found in the lab!

The victim, Mr Blue, was known to have a heart condition but on the bench at the scene where the victim had been working a large bottle of concentrated acid had been upturned and spilt. All around this acid were different chemical bottles which also had been knocked over and may have mixed with the acid. A medicine bottle was also present with unknown tablets inside (Sample X - the tablets have been ground ready for analysis).

Objective

Try to establish cause of death by using infrared analysis to discover the functional groups present in the chemical samples collected. Decide if any of these are likely to be toxic or may have formed a lethal toxic gas on contact with the spilt acid. Establish the identity of the medicine found by library comparison of the spectra and suggest possible implications.

Method

You are provided unknown samples A – H and medicine sample X

1. Run a liquid film on all liquid samples.

2. Use the ATR attachment to run all solid samples.

(Note: Care must be taken with this expensive and fragile equipment, use only when supervised by a demonstrator).

Interpretation of Spectra

To interpret the spectra obtained from a sample it is necessary to refer to correlation charts and tables of infrared data. There are many different tables available for reference, one of which has been provided.

3. Using the correlation chart provided interpret your spectra and identify the functional groups present in the chemical.

Record your results in the table provided.

Identification of Unknown Compound

While IR spectroscopy is a very useful tool for identifying the functional groups in an unknown compound, it does not provide sufficient evidence to confirm the exact structure. Chemists make use of a variety of techniques in order to piece together the structure of a molecule.

4. Use your interpreted IR spectra and the mass spectra provided to determine the structure of all unknown compounds.

5. Identify any chemicals that you think may be toxic or where the functional group may possibly release a toxic gas on contact with an acid.

6. Suggest what other instrumental technique or techniques would be required to confirm the identity of the chemicals. (Your demonstrator will then be able to provide you with additional data for confirmation of analysis).

7. Identify sample X by using the library spectra.

Identification of unknown compounds

Use interpreted IR spectra and mass spectra below to determine the structure of the unknown compounds

Sample A **Empirical Formula C7H6O2**
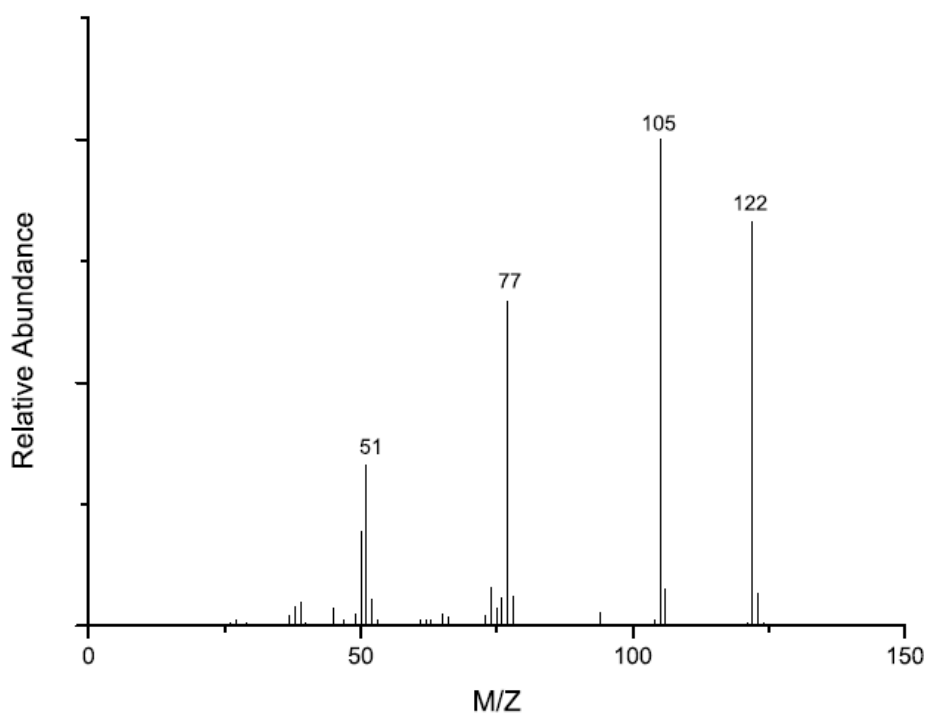
Sample B **Empirical Formula C3H8O**
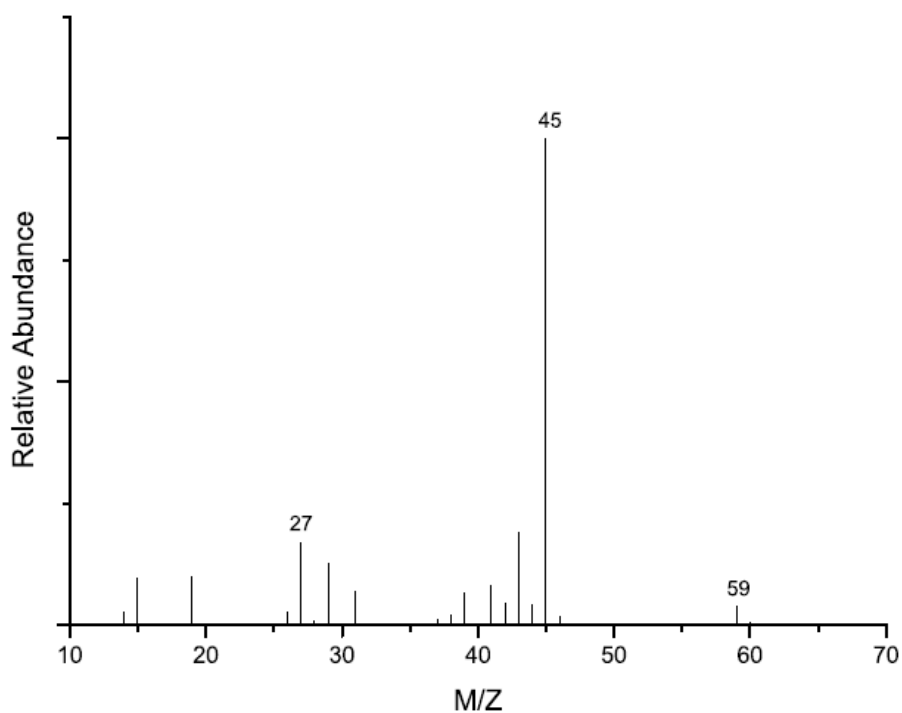
Sample C   **Empirical Formula C3H6O**
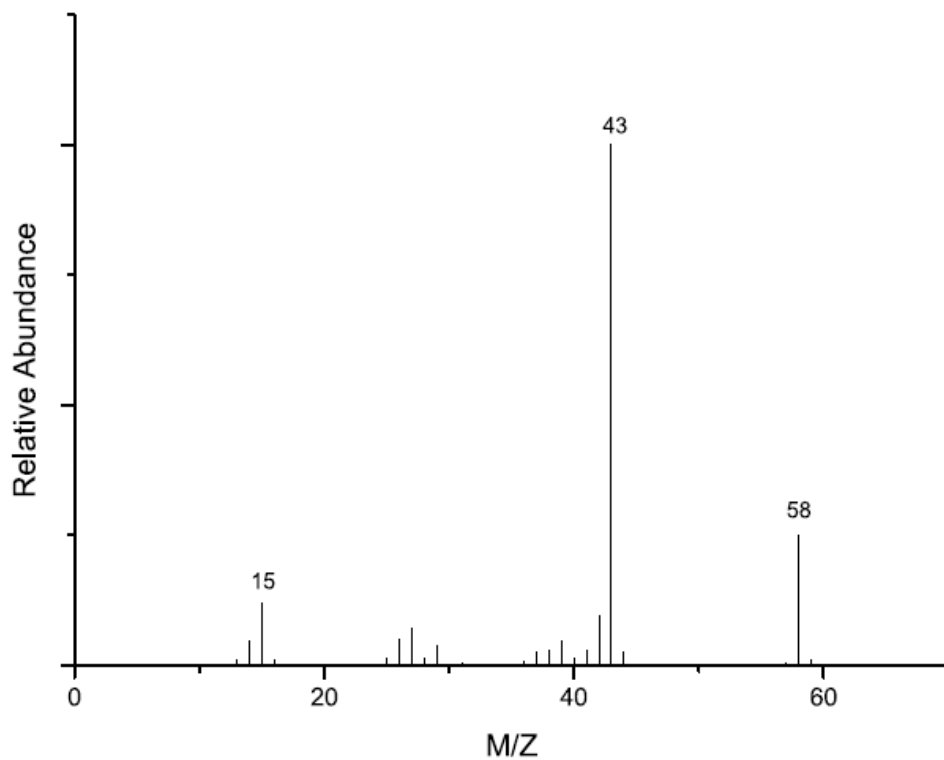
Sample D   **Empirical Formula C9H10O2**
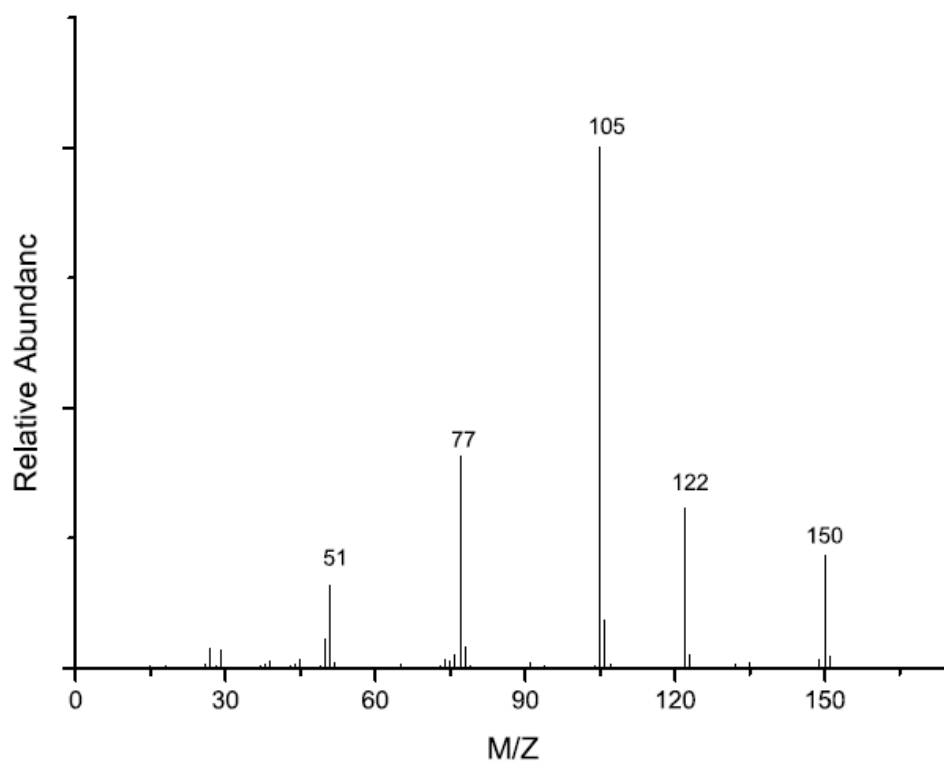
Sample E

Empirical Formula C2H3N
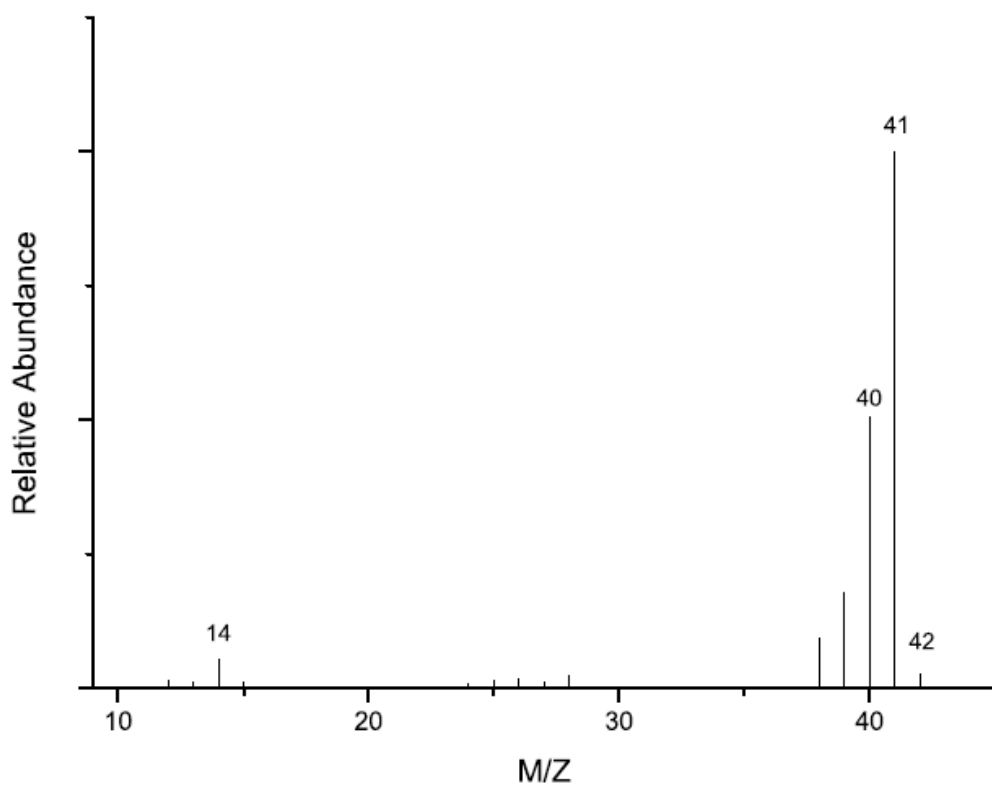
Sample F

Empirical Formula C3H7NO
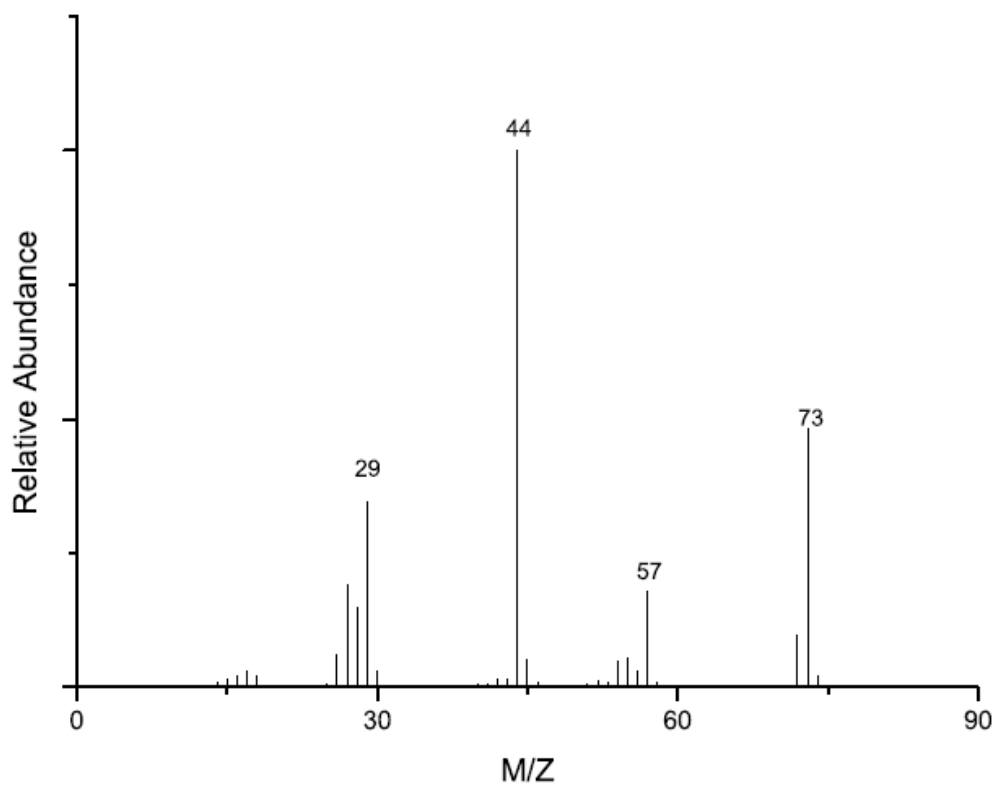
Sample G

Empirical Formula C7H6O
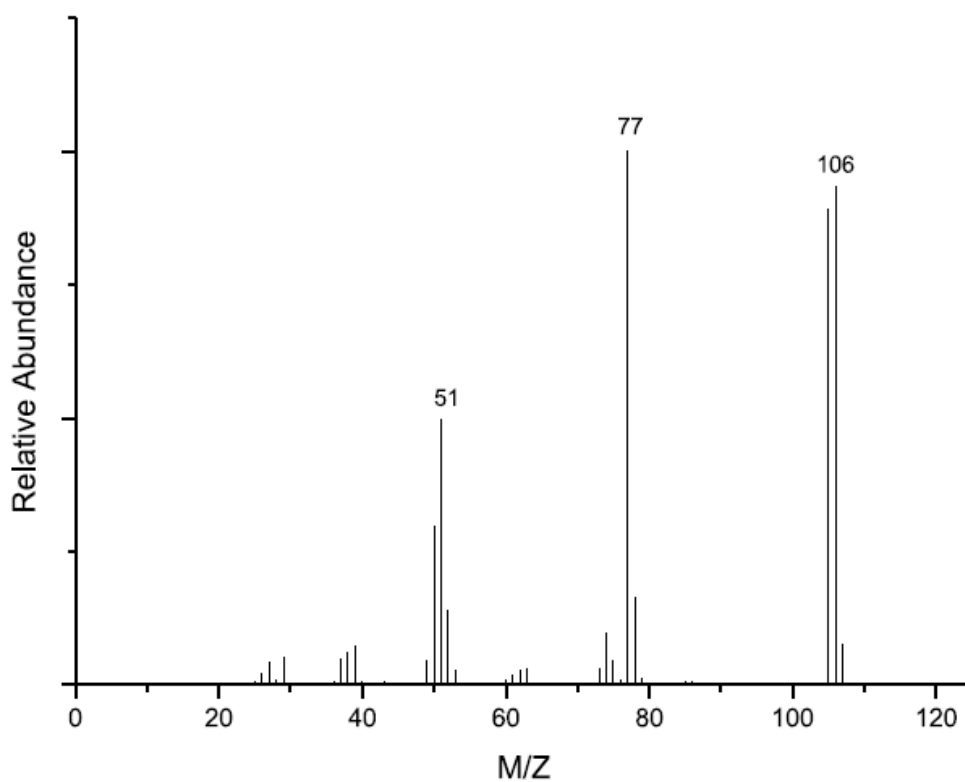
Sample H

Empirical Formula C2H6O
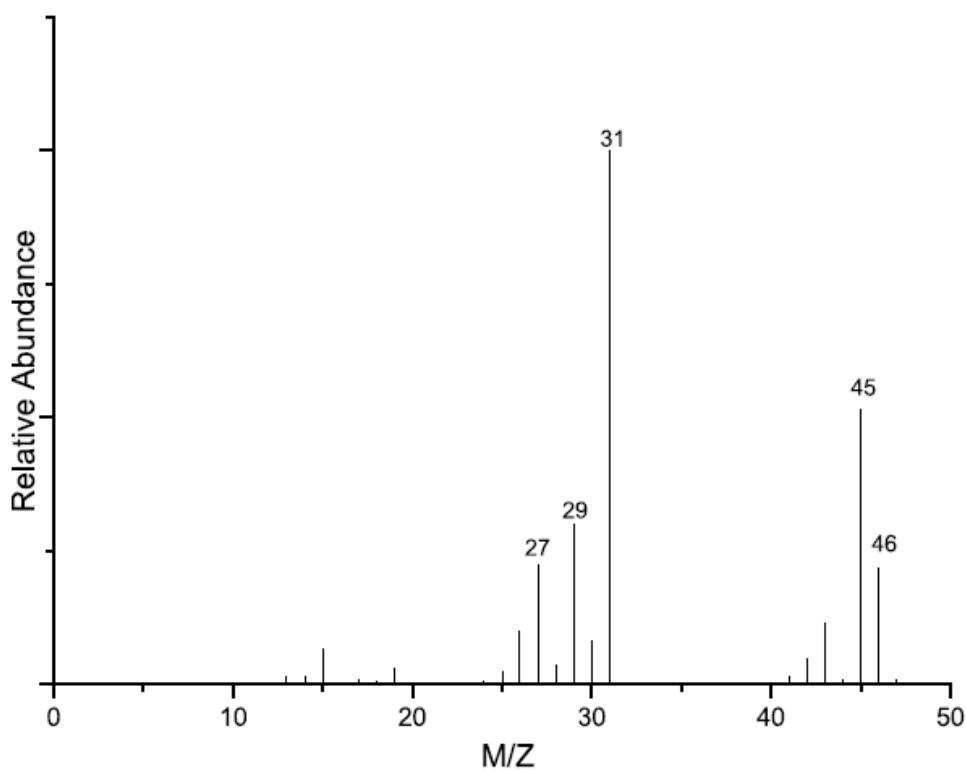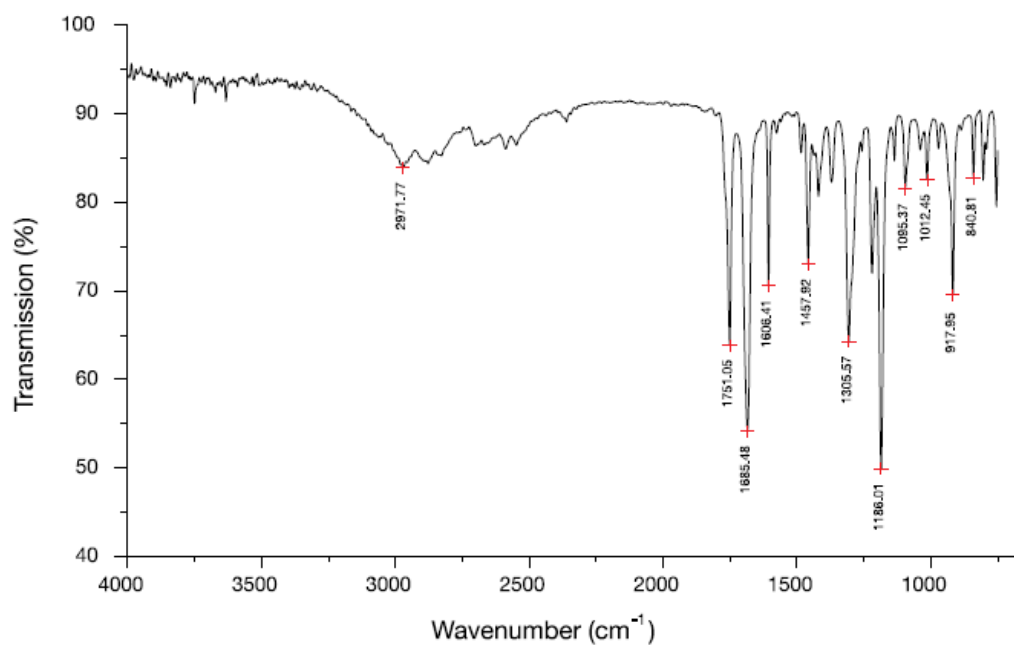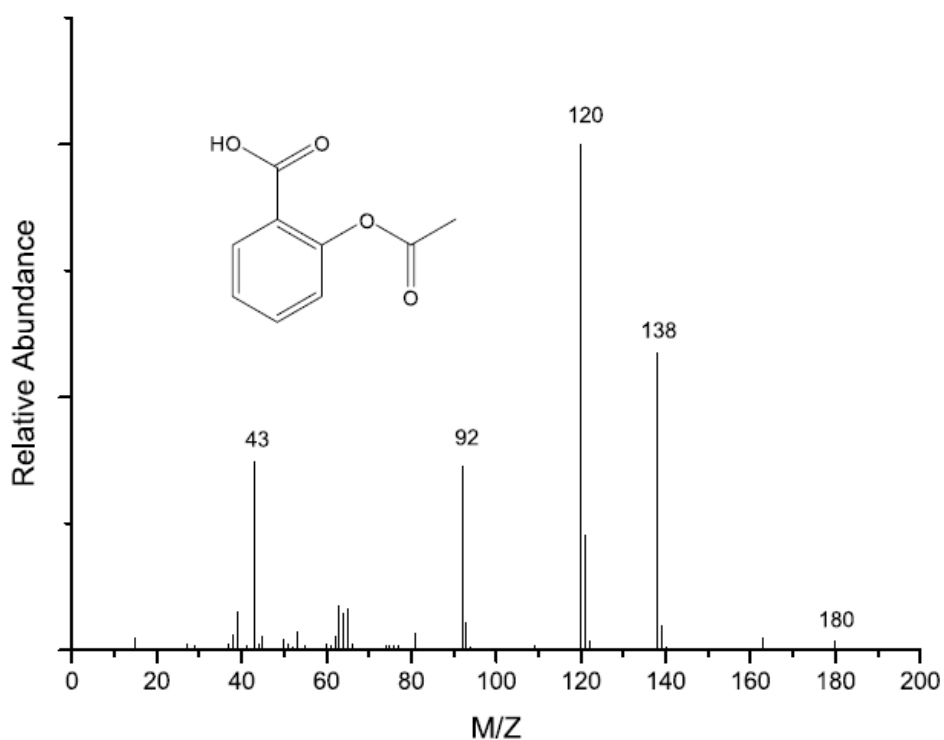
RESULTS

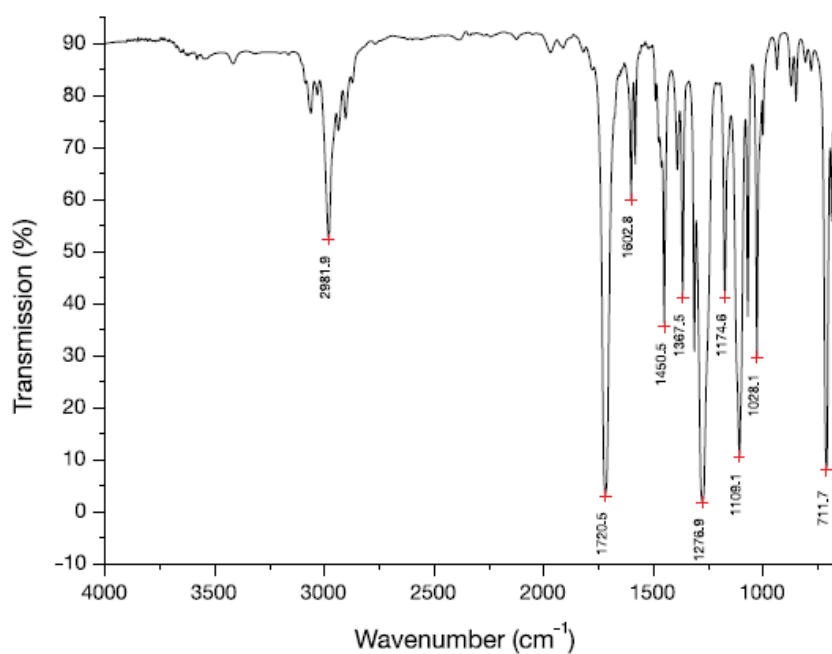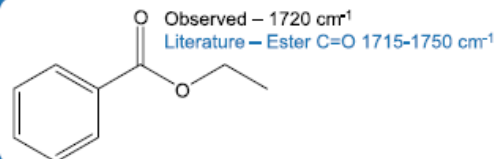| SAMPLE	IMPORTANT PEAK VALUES (cm⁻¹)	FUNCTIONAL GROUP AND RANGE (cm⁻¹)	CLASS OF CHEMICAL (acid, alcohol, Ketone, aldehyde, ester, amide, nitrile)	NAME OF CHEMICAL (Using additional information)
A	2500-3000 1695	OH 2500-3000 C=O 1670-1720	Aromatic Acid	Benzoic Acid
B	3350	OH 3000-3400	Alcohol	Propan-2-ol
C	1714	C=O 1665-1725	Aldehyde or Ketone	Propanone
D	1720	C=O 1715-1750	Ester, Aldehyde or Ketone	Ethylbenzoate
E	2252	C≡N 2220-2260	Nitrile	Acetonitrile
F	3187-3360 1660	N-H 3100-3450 C=O 1630-1690	Amide	Propanamide
G	1701	C=O 1680-1725	Aromatic Aldehyde, Ketone or Ester	Benzaldehyde
H	3331	OH 3000-3400	Alcohol	Ethanol
X	2500-3000 1750 1683	OH 2500-3000 Acid C=O Aryl Ester C=O 1690-1720	Aromatic Acid *Complex sample other groups present	Acetylsalicylic Acid (Aspirin)

MODEL SPECTRA

Sample A – Benzoic Acid

Observed – 1695 cm⁻¹
Literature – Acid C=O 1670-1720 cm⁻¹

Observed – 2500-3000 cm⁻¹
Literature – Acid OH 2500-3000 cm⁻¹

Sample B – Propan-2-ol

Observed – 3350 cm⁻¹
Literature – Alcohol 3000-3400 cm⁻¹

Sample C – Propanone

Observed – 1714 cm⁻¹
Literature – Ketone C=O 1665-1725 cm⁻¹

Sample D – Ethylbenzoate

Observed – 1720 cm⁻¹
Literature – Ester C=O 1715-1750 cm⁻¹

Sample E – Acetonitrile

Observed – 2252 cm⁻¹
Literature – Nitrile CN 2220–2260 cm⁻¹

Sample F – Propanamide

Observed – 1656 cm⁻¹
Literature – Amide C=O 1630–1690 cm⁻¹

Observed – 3187–3360 cm⁻¹
Literature – Amide NH 3100–3450 cm⁻¹

Sample G – Benzaldehyde

Observed – 1701 cm⁻¹
Literature – Aldehyde CO 1680-1725 cm⁻¹

Sample H – Ethanol

Observed – 3331 cm⁻¹
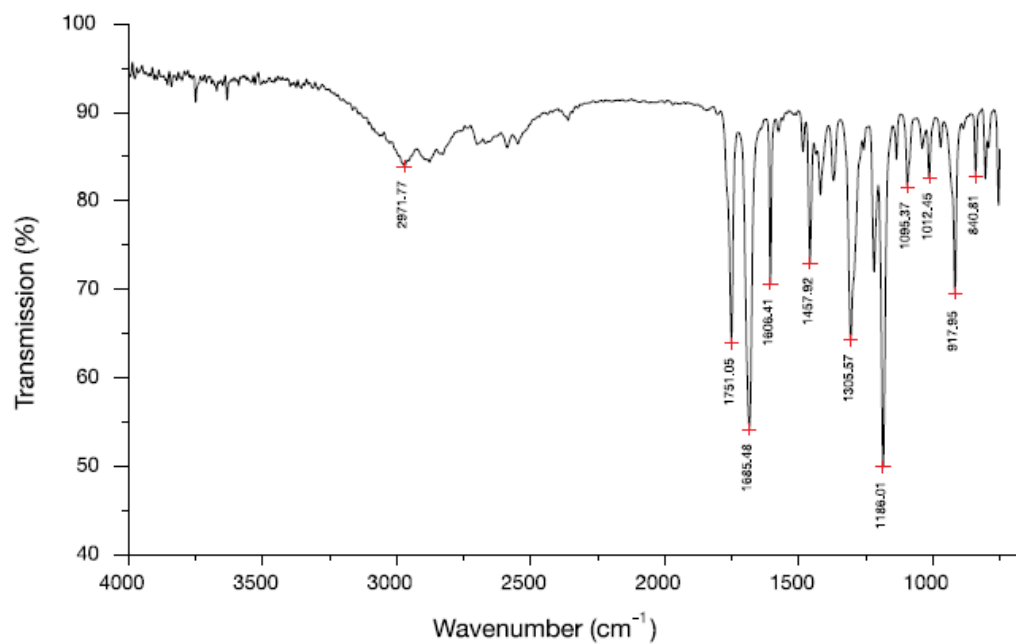Literature – Alcohol OH 3000-3400 cm⁻¹

Sample X – Acetylsalicylic Acid (Aspirin)

Observed – 1683 cm^{-1}
Literature – Acid C=O 1690-1720 cm^{-1}

Observed – 2500-3000 cm^{-1}
Literature – Acid OH 2500-3000 cm^{-1}

Observed – 1750 cm^{-1}
Literature – Ester C=O 1715-1750 cm^{-1}

Peaks: 2971.77, 1751.05, 1685.48, 1606.41, 1457.92, 1305.57, 1186.01, 1095.37, 1012.45, 917.95, 840.81

STUDENT WORK SHEET

SAMPLE	IMPORTANT PEAK VALUES (cm⁻¹)	FUNCTIONAL GROUP AND RANGE (cm⁻¹)	CLASS OF CHEMICAL (acid, alcohol, Ketone, aldehyde, ester, amide, nitrile)	NAME OF CHEMICAL (Using additional information)
A				
B				
C				
D				
E				
F				
G				
H				
X				

Activity 4:Body in a Lab: Compound determination

Technician notes

Chemicals

Aspirin tablets (**harmful)** See CLEAPSS Hazcard 52
Paracetamol tablets
Ibruprofen tablets

~1 ml each
Unknown Samples

A	Benzoic Acid	Acid
B	Propan-2-ol	Alcohol
C	Propanone	Ketone
D	Ethylbenzoate	Ester
E	Acetonitrile	Nitrile
F	Propionamide	Amide
G	Benzaldehyde	Aldehyde
H	Ethanol	Alcohol
X	Acetylsalicylic Acid	Acid

Apparatus

- FTIR infrared spectrometer with ATR attachment and plate holder
- 4 sets sodium chloride plates
- Disposable pipettes and teats
- 2 x tissues
- Acetone wash bottle (**Highly flammable, irritant**) See CLEAPSS Hazcard 85
- Propan-2-ol wash bottle (**Highly flammable, irritant**) See CLEAPSS Hazcard 84A
- 2 x micro-spatula

Administration

- Poster
- Scripts
- 5 x laminated correlation charts
- Sample spectra for IR and mass spec.
- Pens

Glossary

Absorbance	The amount of incident light at a set wavelength absorbed as it passes through a sample.
ATR	Attenuated total reflectance – a sample preparation system for FTIR
Fourier transform	A mathematical operation that decomposes a signal into its constituent frequencies.
Resonance	Resonance is the tendency of a bond to oscillate at a greater amplitude at some frequencies than at others.
Transmittance	The amount of incident light at a set wavelength that passes through a sample.