MODERN ANALYTICAL CHEMISTRY

12

A1 Analytical techniques
A2 Principles of spectroscopy
A3 Infrared (IR) spectroscopy
A4 Mass spectrometry
A5 Nuclear magnetic resonance (NMR) spectroscopy
A9 Nuclear magnetic resonance (NMR) spectroscopy (HL)
A6 Atomic absorption (AA) spectroscopy
A7 Chromatography
A10 Chromatography (HL)
A8 Visible and ultraviolet spectroscopy (HL)

"Analytical chemistry techniques are widely used in today's society. Students should understand the chemical principles behind each analytical technique. This option builds on some of the key ideas in both physical and organic chemistry that were introduced in the core".

A1 ANALYTICAL TECHNIQUES

A1.1 State the reasons for using analytical techniques.

A1.2 State that the structure of a compound can be determined by using information from a variety of analytical techniques singularly or in combination.

Analytical chemistry involves qualitative and quantitative analysis of a sample to determine its chemical composition and structure. **Qualitative analysis** determines what components are present, for example, the presence of a forbidden colouring material in a processed food or the elements present in a compound. **Quantitative analysis** determines the amount of a particular substance in a mixture, for example the percentage of copper in brass or the amount of each element in a compound. **Structural analysis** is the determination of the structure of a pure substance, the way in which the atoms present are joined together and, in the case of large molecules, the way in which the molecule is arranged in three-dimensions as in the structure of a protein.

'Wet' chemical techniques involve observing characteristic chemical reactions, or the use of volumetric and gravimetric techniques. Analytical methods, on the other hand, are usually faster, more precise and easier to automate than 'wet' methods, and a combination of analytical techniques is often used to obtain complete structural information. Thus, whereas an IR spectrum shows the presence of organic functional groups such as carbonyl or hydroxyl groups, NMR is more diagnostic and can help determine molecular structure. GC-MS uses gas chromatography to separate a mixture of compounds followed by identification using mass spectroscopy.

A2 PRINCIPLES OF SPECTROSCOPY

A2.1 Describe the electromagnetic spectrum.

Many analytical techniques involve spectroscopy, i.e., the way in which the **absorption** or **emission** of **electromagnetic radiation** by substances varies with frequency**. Electromagnetic radiation** ranges from very high energy γ-rays through to low energy radio waves and beyond. A wave is characterized by its **wavelength** or **frequency**. The wavelength (λ, in units of distance, e.g.,

OPTION

m) is the distance between successive peaks on the wave (Figure 1201). The frequency (f, in units of Hertz, Hz = s^{-1}) is the number of peaks that pass a fixed point every second. All electromagnetic waves travel at the same speed in a vacuum ($c = 3.00 \times 10^8$ m s^{-1}), and the relationship between frequency and wavelength:

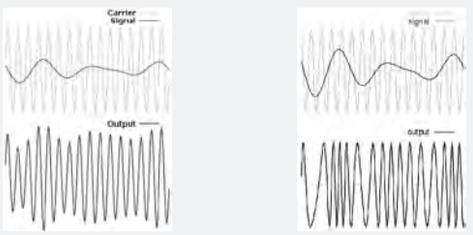
Velocity of light = frequency × wavelength ($c = f\lambda$)

holds for ***all*** electromagnetic waves.

TOK How is the nature of the information carried by the electromagnetic spectrum limited by its wavelength?

Now this is a little bit different. In these things I'm used to discussing the boundaries between belief and knowledge, but maybe this one is more the boundary between TOK and Physics? I can think of a couple of ways in which this is true. Firstly there is the factor relevant to considering whether we could ever 'see' an atom. In order to see something, the electromagnetic waves have to be able to focus on an image of the object and this means that the wavelength of the waves has to be comparable to, or less than, the size of the object. Visible light has a much longer wavelength than the size of atoms so we can no more see atoms (until we develop γ-ray sensitive eyes) than we can take a family photo using radio waves.

Secondly there is the use of waves to transmit information. A constant wave transmits nothing, so to send a message it has to be altered in some way. The two most common ones are amplitude modulation (AM - altering how loud it is in a sound analogy) or frequency modulation (FM – altering how high pitched it is); these are illustrated below:



In both cases the signal is imposed on top of the 'carrier' wave, which must be of a shorter wavelength than the 'modulation' which carries the information, so that the shorter the wavelength, then the greater the range of frequencies available for transmitting information. I suppose it is going to be like that until we find a smarter way of sharing information.

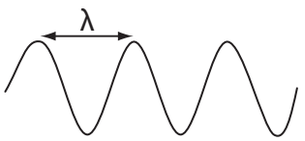


Figure 1201 An electromagnetic wave

Electromagnetic radiation also has a particle nature and each photon (particle of light) carries a quantum of energy. The energy of the quantum of radiation is related to the frequency of the radiation by the equation:

$$E = \frac{hc}{\lambda} \text{ or } E = hf$$

(where h = Planck's constant, 6.626×10^{-34} J s)

Example

If a molecule absorbs IR radiation of λ = 900 nm, calculate the energy absorbed per mole.

Solution

$$\Delta E = hf = \frac{hc}{\lambda} = \frac{6.63 \times 10^{-34}\,\text{J s} \times 3.00 \times 10^{8}\,\text{m s}^{-1}}{900 \times 10^{-9}\,\text{m}}$$

$= 2.21 \times 10^{-19}$ J per molecule

∴ Energy absorbed per mole

$= 2.21 \times 10^{-19}\,\text{J} \times 6.02 \times 10^{23} = 1.33 \times 10^{5}\,\text{J mol}^{-1} = 133\,\text{kJ mol}^{-1}$.

Thus high frequency (and hence short wavelength) radiation carries a great deal of energy and radiation of low frequency carries much less. A particle (atom, molecule or ion) can absorb a quantum of light and this will affect its state. The way in which its state is affected will depend on the amount of energy that the quantum carries:

- **γ-rays**, the highest frequency radiation, can bring about changes in the nucleus.
- **X-rays** cannot cause changes in the nucleus, but have enough energy to remove electrons in inner filled shells of atoms.
- **Ultraviolet** and **visible light** have enough energy to affect the valence electrons.

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- **Infrared** radiation, perceived as heat, can stimulate the vibrations of molecules.
- **Microwaves** affect the rotational state of molecules.
- **Radio waves** can alter the spin state of some nuclei when they are exposed to magnetic fields and are used in NMR spectroscopy.

Exposure to high intensities of any types of radiation can be harmful to health, but γ-rays, X-rays, and UV can break chemical bonds and initiate reactions; hence, they are harmful even at low intensities. UV radiation in sunlight with too much exposure causes sunburn. See Figure 1202 for more information about the electromagnetic spectrum, and the changes it causes.

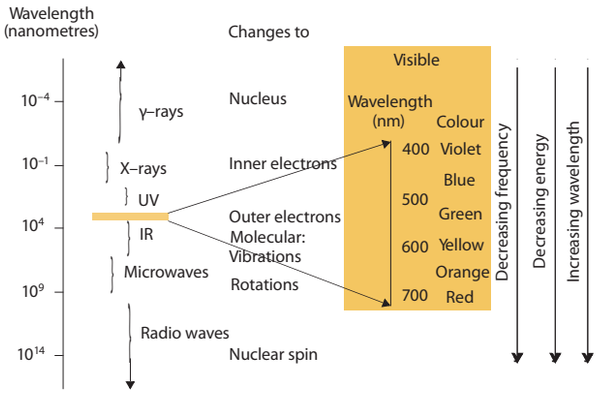

Figure 1202 The Electromagnetic Spectrum

A2.2 Distinguish between *absorption* and *emission* spectra and how each is produced.

In **emission spectroscopy** the frequency of the radiation emitted by excited particles dropping to a lower energy state is studied, e.g., the coloured light from a neon lamp is an emission process, as is the emission line spectrum of hydrogen see Figure 1203(a). In **absorption spectroscopy**, radiation of a wide range of frequencies is passed through the sample and the intensity of the radiation of various frequencies emerging on the other side is compared to that going in, to find out which frequencies are absorbed by the sample. Energy of particular frequencies is absorbed and used to enable a particle to move from a lower to a higher energy state, see Figure 1203(b). The red colour of red paint is the result of an absorption process because, of the wide range of frequencies in the white light shining on it, the paint absorbs the blue, green and yellow colours and it reflects the red light. Thus it is the absorption of light in the visible range that makes things coloured.

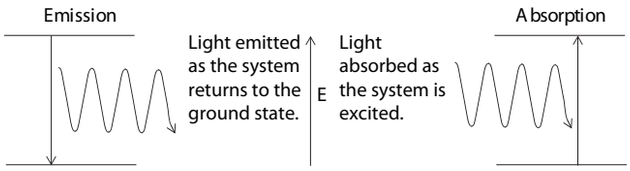

Figure 1203 (a) and (b) Emission and absorption spectroscopy

Exercise A2

1. Which of the following types of radiation has quanta of the highest energy?

   A X-rays
   B UV light
   C IR light
   D Microwaves

2. Green light has a wavelength of 500 nm. What is the frequency of this light?

   A 0.002 Hz
   B 3.31×10^{-31} Hz
   C 7.55×10^{35} Hz
   D 6.00×10^{14} Hz

3. Consider the following techniques:

   gas–liquid chromatography
   NMR spectroscopy
   mass spectrometry
   column chromatography
   IR spectroscopy
   UV–visible spectroscopy

   For each of the following problems, state which of the above techniques would be the most appropriate and justify your choice.

   a) Determining the concentration of an aqueous solution of copper(II) sulfate.
   b) Detecting the presence of 2–methylheptane in petrol.
   c) Whether a sample is propan–1–ol or propan–2–ol (assume no pure samples or data on these is available).
   d) Obtaining a pure sample of pure 4–nitrobenzene from a mixture with 2–nitrobenzene.
   e) Assessing the ^{16}O to ^{18}O ratio in a sample of ice from Antarctica.

OPTION

4. Modern analytical techniques have had a great impact in many other fields, but probably the greatest has been upon the medical sciences. Discuss three examples where three different techniques have contributed to the medical sciences and describe how their introduction has led to improvements.

A3 INFRARED SPECTROSCOPY

A3.1 Describe the operating principles of a double beam spectrometer

Many measurements in absorption spectroscopy employ **double-beam** instruments that allow the radiation passing through the sample to be compared with identical radiation that has not passed through the sample. The radiation from the source is split into two equal beams that pass along parallel paths. The sample is placed in one beam, whilst the second, known as the **reference**, is identical containing the same cell and solvent but without the substance being studied. The light from the source passes through a **monochromator**, which only allows radiation of a particular wavelength to pass through it. This **monochromatic light** (light of a single colour or single wavelength) then strikes a beam-splitter, which directs half of the radiation through the sample and the other half through the reference cell. The two beams are then recombined at the detector. The signals from the sample and reference beams are then compared electronically to see if the sample absorbs radiation of the frequency that the monochromator is set to and the output is sent to the recorder. As the spectrum of the sample is scanned, the frequency of the radiation that the monochromator transmits is varied and a graph of absorption against frequency (or wavelength or wavenumber) is drawn. Comparison of the spectrum of the unknown compound with a data bank enables its identification. The principle of the double-beam instrument (in UV-visible or infrared: they differ only in the source and detector) is illustrated in Figure 1204.

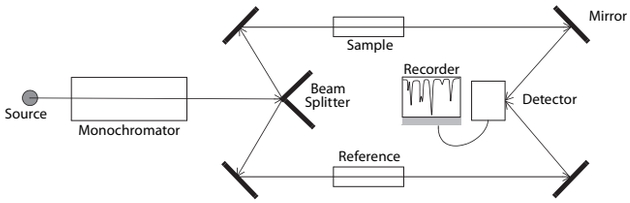

Figure 1204 A double-beam spectrometer

A.3.2 Describe how information from an IR spectrum can be used to identify bonds

The infrared region extends from about 600 cm^{-1} to 4000 cm^{-1}. A quantum of infrared radiation does not have sufficient energy to excite an electron to a higher energy level, but it does have sufficient energy to excite a molecule to a higher vibrational level. There are two types of vibrational motions that most molecules are capable of: stretching motions, where the bond lengths become longer then shorter, and bending motions, where the length of the bonds stays constant, but the angle between them increases and decreases. This latter kind of motion is, of course, not possible in diatomic molecules (such as H-Cl). The stretching and bending motions for water are shown in Figure 1205.

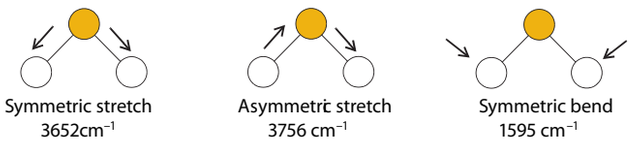

Figure 1205 The bending and stretching motions of water molecules

The 'wavenumbers' at which these motions absorb infrared radiation are shown under each mode. In infrared spectroscopy, the wavenumber (= 1/wavelength, units = cm^{-1}) is used rather than frequency. It is equal to the number of wave peaks in 1 cm of the wave. For example if infrared radiation has a wavelength of 2000 nm (0.002 cm) then its wavenumber = 1/0.002 = 500 cm^{-1}. The greater the wavenumber, the lower the wavelength, the higher the frequency and the greater the energy ($E = hf$). Note therefore that the stretching motions generally require more energy and therefore occur at a greater wavenumber than bending motions.

OPTION

A.3.3 Explain what occurs at a molecular level during the absorption of IR radiation by molecules.

In order to absorb infrared light, a vibrational motion must result in a change in the **dipole moment** of the molecule. Hence diatomic molecules with only one element such as H_2 and O_2 do not absorb infrared radiation. However hydrogen chloride is a polar molecule ($^{\delta+}H - Cl^{\delta-}$) and as the bond stretches, the distance between the atoms increases and so the dipole moment, which depends on both the partial charges and their separations, also increases. Hence the vibration of this bond absorbs infrared radiation of a particular wavenumber (2990 cm^{-1}).

All of the vibrations of water (see Figure 1205) lead to a change in dipole moment and hence to infrared absorption. In a symmetrical linear molecule, such as carbon dioxide, however, the symmetrical stretching mode does not change the dipole of the molecule (or, more precisely, it maintains the symmetry that leads to the molecule being non-polar) and hence it does not give rise to an infrared absorption, though other vibrations, such as the asymmetric stretch and the symmetric bend, do affect the dipole and thus absorb infrared radiation, as shown in Figure 1206.

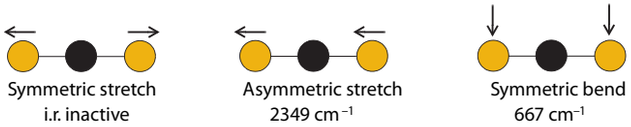

Figure 1206 The bending and stretching motions of carbon dioxide:

Likewise the symmetrical stretching mode of a symmetrical tetrahedral molecule is not infrared active, because it does not result in any change of the dipole of the molecule. Sulfur dioxide is non-linear (due to the effect of non-bonding e-pair) and similar in shape to water (bent). Hence, in contrast to carbon dioxide, even the symmetric stretch causes a change in dipole moment and is infrared active.

A.3.4 Analyse IR spectra of organic compounds *(up to three functional groups).*

The masses of atoms involved and the strength of the bond determines its infrared absorption frequencies, with heavier atoms and stronger bonds requiring more energy (see Figure 1207 and also the IBO Chemistry Data Book). Indeed a careful study of the infrared absorption frequency can be used to calculate the strength of the bond between two atoms.

Bond	Bond enthalpy / kJ mol^{-1}	Wavenumber / cm^{-1}
C – C	348	800 – 1200
C = C	612	1610 – 1680
C ≡ C	837	2070 – 2250
C – H	412	2840 – 3095

Figure 1207 Bond enthalpies and wavenumbers

With many bonds, the mass of the parts of the molecule attached by the bond (or at least the ratio of the mass of the lighter part to the rest, which is more important) tend not to vary too much and so absorptions involving that bond tend to be in a particular region of the infrared spectrum. Hence absorption of radiation of this frequency indicates the presence of this bond in a molecule. This is of particular use in deducing the structure of organic molecules. The precise wavenumber depends to some extent on the other groups present, so a range of frequencies is associated with that bond, as indicated in Figure 1208.

Bond; Organic molecules	Wavenumber / cm^{-1}
C – Cl: halogenoalkanes	700 – 800
C – O: alcohols, ethers, esters	1000 – 1300
C = C: alkenes	1610 – 1680
C = O: aldehydes, ketones, acids, esters	1680 – 1750
C ≡ C: alkynes	2070 – 2250
O – H: H-bonded in –COOH	2500 – 3300
C – H: alkanes, alkenes, arenes	2840 – 3095
O – H: H-bonded in alcohols, phenols	3230 – 3550
N – H: primary amines, RNH_2	3350 – 3500

Figure 1208 The IR absorption wavenumbers of some bonds

Particularly useful is the very strong absorption at 2550-3230 cm^{-1} due to the –OH group in alcohols and carboxylic acids, and the carbonyl group (>C=O) absorption found at 1680-1750 cm^{-1} in aldehydes, ketones, esters and carboxylic acids (see the spectrum of ethanoic acid in Figure 1209). Since the alcohol content of the breath is related to its content in the blood, infrared absorption due to C-H vibrations in the range 2840-3095 cm^{-1} is used in the 'intoximeter' to determine whether motorists have an illegal level of alcohol. (Note the 3250-3550 cm^{-1} peak due to O-H cannot be used since moisture in the breadth would also absorb in this region.) Absorption by >C=C< in the 1610-1680 cm^{-1} region can also be used to assess the degree of unsaturation present in vegetable oils.

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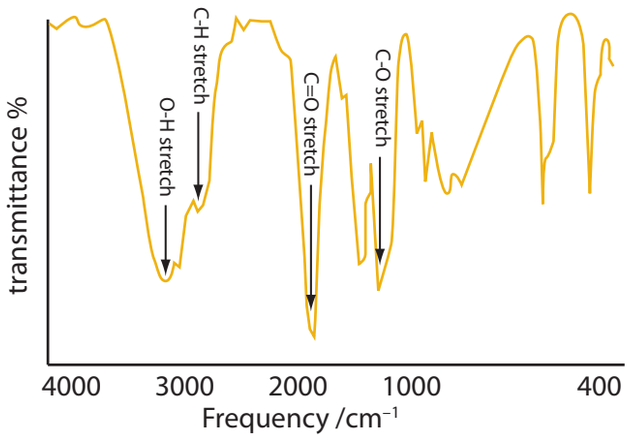


Figure 1209 IR Spectrum of ethanoic acid

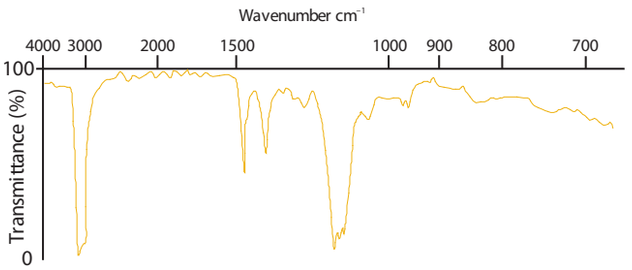
Infrared spectra tend to be complex due to the large number of vibrations possible, hence an infrared spectrum, especially in the region 500 cm^{-1} to 1500 cm^{-1} (sometimes called the **'fingerprint' region**) is unique to that compound. This means that a comparison with standard spectra in a library can often be used to identify an unknown compound.

Exercise A3

1. A species has an infrared absorption at 2000 cm^{-1}. What is the wavelength of the light?

   A 2×10^{5} m
   B 20 m
   C 5×10^{-2} m
   D 5×10^{-6} m

2. Consider the IR absorption spectrum of a compound, given below. Use a table of infrared absorption frequencies to assign two of the peaks. Also identify two groups that are not present in the compound.



   Explain why, given access to a library of IR spectra, this could be used to identify the compound.

3. Boron trifluoride, BF_3, is a trigonal planar molecule that absorbs radiation in the infrared region of the spectrum:

   a) What changes in the molecule lead to it absorbing in this spectral region?
   b) Not all changes of this type are infrared active. Explain why this is so.
   c) Use sketches to illustrate one that would be IR active and one that would not.

4. The positions of absorption bands in IR spectra are usually quoted in wavenumbers, with units of cm^{-1}. How is this related to the frequency of the radiation? Water absorbs radiation at 3652 cm^{-1}. Calculate the wavelength and frequency of this radiation?

A4 MASS SPECTROMETRY

A.4.1 Determine the molecular mass of a compound from the molecular ion peak.

In the **mass spectrometer**, gaseous molecules are converted to positive ions and these ions, after being accelerated through an electric field, are deflected by a magnetic field. The lower the mass of the ion, the greater the deflection and so, by varying the strength of the magnetic field, ions of differing mass can be brought to focus on the detector. The mass spectrum records the relative abundances of the fragments of different mass reaching the detector. To be more precise the **mass/charge (m/z) ratio** is measured, though as conditions are chosen to primarily generate singly charged ions, it is common just to refer to it as the 'mass'.

The inside of the mass spectrometer is at high vacuum so that the ions cannot collide. Hence, the ion with the greatest mass will usually correspond to a molecule that has only lost a single electron – the molecular ion. The mass of the molecular ion gives the relative molecular mass of the molecule. This can be combined with data from elemental analysis to calculate the molecular formula of the substance. In some modern instruments the relative molecular mass of the molecular ion can be found to such

precision that, using the fact that relative atomic masses of isotopes are not precise integers (e.g. ^{16}O is 15.995), the molecular formula can be calculated directly as to this precision, for example, CO (M_r = 27.995) , N_2 (M_r = 28.0062) and C_2H_4 (M_r = 28.032) have different relative molecular masses.

A.4.2 Analyse fragmentation patterns in a mass spectrum to find the structure of a compound.

The excess energy from the impact of the electron forming the molecular ion will often cause it to break down, or 'fragment', inside the mass spectrometer giving rise to a '**fragmentation pattern**' of lower molecular mass ions. This fragmentation pattern can be used for 'fingerprint' purposes (see infrared spectra above), to allow the identification of the molecule by comparison with the spectra of known compounds from a library. The mass of the units that have broken off the molecule will frequently give clues as to the structure of the molecule. Sometimes only a hydrogen will break off, giving a peak at one mass number less than the main peak. If two fragments differ in mass by 15 then this probably corresponds to the loss of a methyl (CH_3–) group. Similarly a loss of 17 corresponds to the loss of HO-, 29 to the loss of C_2H_5– or H–CO–, 31 to the loss of CH_3–O– and 45 to the loss of –COOH. Figure 1211 shows the mass spectrum of butane and Figure 1212 that of propanoic acid with the molecular ion and some fragments labelled.

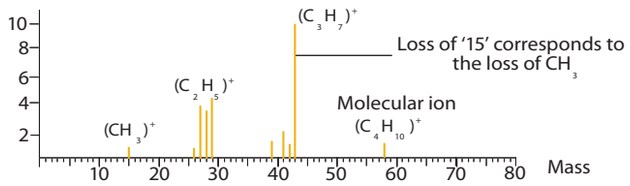


Figure 1211 The Mass spectrum of butane

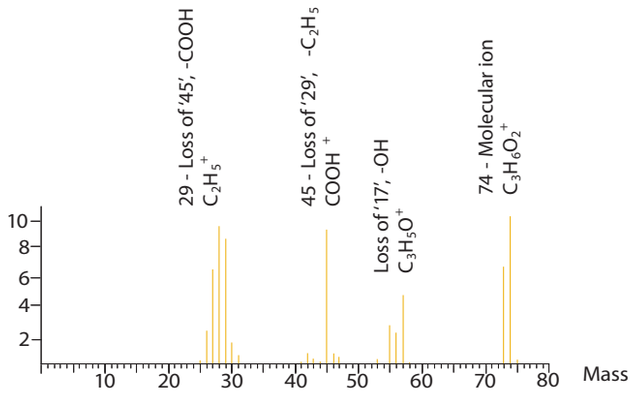


Figure 1212 The Mass spectrum of propanoic acid

Because of the possible places that bonds can break, the spectra of quite similar molecules can often be significantly different as shown in the mass spectra of the two isomers of octane below and the two propanols see Figure 1214:

Mass spectra can be produced from minute (as small as 10^{-6} g) samples. Besides determination of atomic and molar masses and organic structure, it can be used to detect the percentage of ^{14}C present in a sample in the process known as **radiocarbon dating** and in forensic science to determine the presence of small amounts of drugs and other substances of interest.

EXTENSION

A smaller peak is usually found in organic mass spectra at one mass number greater than the main peak. This is due to the presence in the compound of the isotope ^{13}C, which comprises about 1% of naturally occurring carbon. Its size, relative to the main peak, therefore depends on the number of carbon atoms in the molecule, because if there are six carbon atoms there is an approximately 6% chance (6 × 1%) of there being an atom ^{13}C of in the molecule. This peak can clearly be seen in Figure 1212.

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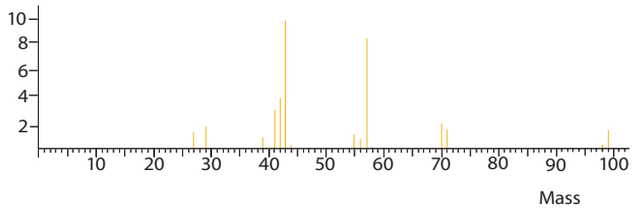
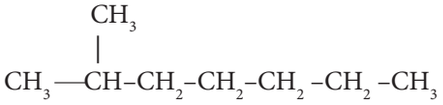
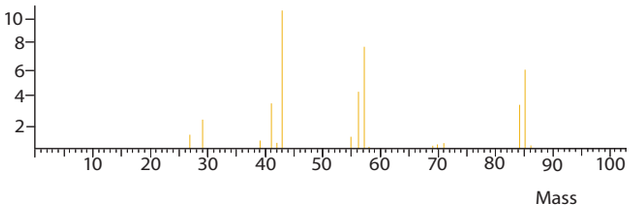
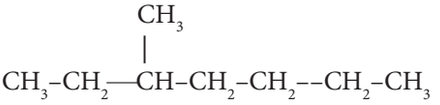




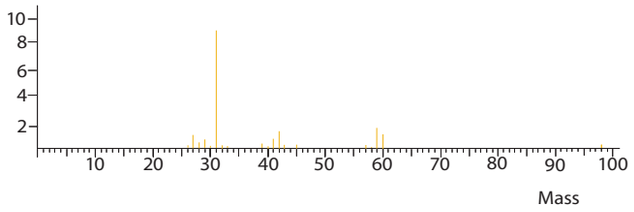
2-methylheptane has a significant fragment at $(M_r - 15)^+ = 99$ due to easy loss of CH_3 at branch on C #2





3-methylheptane has a significant fragment at $(M_r - 29)^+ = 85$ due to easy loss of C_2H_5 at branch onC #3.

Figure 1213 (a) and (b) Comparing the mass spectra of two isomers of octane (C_8H_{18})

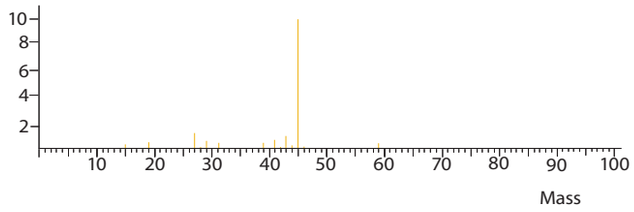


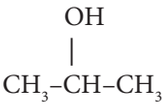
$CH_3–CH_2–CH_2–OH$

Only small peak at 45: $(M_r - 15)^+$: $(CH_2CH_2OH)^+$: due to loss of CH_3, as strong C-C bond to break.

Strong peak at 31: $(CH_2OH)^+$ from $(M_r - 29)^+$: due to loss of C_2H_5.; weaker C-C bond due to inductive effect of –OH.

Peak at 29 due to $(C_2H_5)^+$.





Strong peak at 45: $(M_r - 15)^+$: $(CH_3CHOH)^+$: due to loss of CH_3

No peak at 31: A peak at 31 requires loss of C_2H_5 $(M_r - 29)^+$and propan-2-ol has no C_2H_5 to lose.

Peak at 27 due to $(CH_3C)^+$.

Figure 1214 (a) and (b) Comparing the mass spectra of propan-1-ol and propan-2-ol (C_3H_7OH)

Chlorine and bromine both comprise mixtures of isotopes with a mass difference of two (35 & 37 for Cl, 79 & 81 for Br). So in mass spectra involving these atoms there are two peaks two units apart. Their relative magnitudes reflect the natural abundances of the isotopes (3:1 ^{35}Cl: ^{37}Cl for chlorine, approximately equal abundances for bromine). If there are two halogen atoms present, then there will obviously be three peaks; in a ratio 9:6:1 for chlorine

$$\left(\frac{3}{4}\times\frac{3}{4}\right) : 2\left(\frac{3}{4}\times\frac{1}{4}\right) : \left(\frac{1}{4}\times\frac{1}{4}\right)$$

and 1:2:1 for bromine. These can readily be observed in the mass spectrum of 2-chloropropane in Figure 1215 and of 1.2-dibromoethane in Figure 1216.

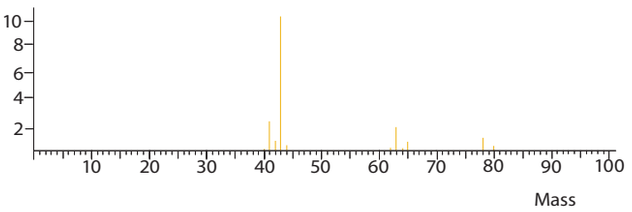


Figure 1215 The mass spectrum of 2-chloropropane

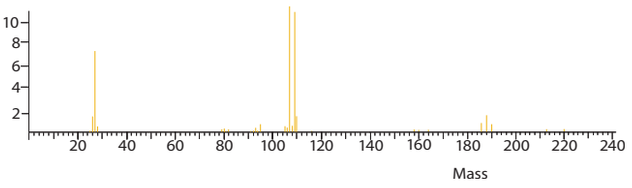


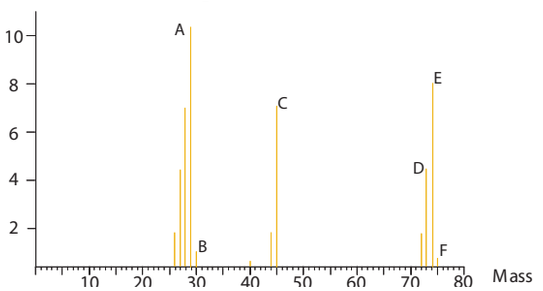
Figure 1216 The mass spectrum of 1.2-dibromoethane

EXTENSION

Exercise

1. a) Determine the empirical formula of a hydrocarbon that contains 83.3% carbon by mass?
   b) Describe how you could use the mass spectrum to find the molecular formula of the compound. Outline how the mass spectrum could be used to confirm that it was indeed a hydrocarbon and not an oxygen/nitrogen containing molecule.
   c) Assume the molecular formula is C_5H_{12}. Consider the possible isomers and the way in which these might split up in a mass spectrometer to produce fragments around 55-57, 40-43 and 25-29. How might you attempt to deduce which isomer you had from the mass spectrum?

2. The mass spectrum below is that of a carboxylic acid.
   a) Identify the carboxylic acid in question.
   b) Give the formulae of the species that give rise to the peaks labelled A to F.



3. (Extension Question). When introduced into a mass spectrometer, dichloroethene gives a distinctive spectrum:

   a) What peaks would you expect to result from the molecular ion? Give the masses you would expect them to occur at and the relative intensities of these peaks.
   b) In what way might you expect the spectrum to reveal whether the dichloroethene is the 1.1 or a 1.2 isomer?
   c) There are two possible 1.2 isomers. Given pure samples of each how could you use a mass spectrometer to differentiate between them. Describe one simpler way of achieving this.

A5 NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

A.5.1 Deduce the structure of a compound given information from its ^{1}H NMR spectrum *(splitting pattern not required at SL)*

Nuclear Magnetic Resonance (NMR) spectroscopy is arguably the most powerful single tool for investigating the structure of a molecule. It is found that, as a result of changes that occur in the nucleus (see Extension for further explanation), atoms with an odd mass number, when placed in a strong magnetic field, absorb radiation of radio frequency. The precise frequency varies slightly with the electron density around the nucleus and hence depends on its chemical environment. Most commonly this is applied to the hydrogen atoms in a molecule. The NMR spectrum indicates the bonding of all of the hydrogen atoms in the molecule and Figure 1218 gives the common bonding situations of hydrogen atoms in organic molecules and the region of the NMR spectrum (called the **chemical shift**, δ, and measured in ppm) that these absorb in.

Bonding situation (R – alkyl group, ⬡ – benzene ring)	Chemical shift, δ ppm
R – CH_3	0.9
R – CH_2 – R	1.3
R_3C – H	2.0
CH_3 – CO – O – R	2.0
CH_3 – CO – R	2.1
⬡–CH_2	2.3
R – C≡C – H	2.6
R – CH_2 – F/Cl/Br/I	3.2 – 3.7
R – O – CH_3	3.8
R – CO – O – CH_2 – R	4.1
⬡–O – CO – CH_3	4.0 – 4.2
R – O – H	0.5 – 6.5
R – CH = CH_2	4.9 – 5.9
⬡–O – H	7.0
⬡–H	7.3
R – CO – H	9.7
R – CO – O – H	11.5

Figure 1218 Characteristic ^{1}H NMR absorptions

From the relative intensities of the signals, the number of hydrogen atoms that are bonded in each of these environments can be determined. This is often displayed as an 'integration trace' (it integrates the area under the peak) in which the length of the vertical section at each peak is proportional to the number of hydrogen atoms in that chemical environment. Knowing the way in which all of the hydrogen atoms are bonded, along with the relative numbers of these (given by the integration trace), will frequently allow the structure of the molecule to be determined without reference to other techniques.

Figure 1219 below shows the low resolution NMR spectrum of ethanol with an interpretation of the various peaks in it. In high resolution spectra (Figure 1222) these broad peaks may appear as groups of separate sharp peaks.

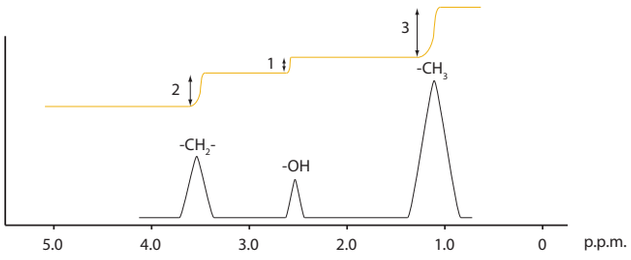


Figure 1219 The low resolution NMR spectrum of ethanol

OPTION

A.5.2 Outline how NMR is used in body scanners *(an important application of NMR Spectroscopy).*

The body scanner also operates on the principle of NMR spectroscopy. The main constituents of the body that contain hydrogen atoms, and hence produce signals, are water and lipids. Different parts of the body have different water-lipid ratios in the tissue and therefore absorb radio frequency radiation in different ways. The patient is placed in a strong magnetic field and as the scanner is moved around the body data about the absorption at various angles can be accumulated to allow a three–dimensional image of the various organs to be built up. The advantage of this technique, called **Magnetic Resonance Imaging** (MRI), in body scanning is obvious as radio waves are harmless. MRI is used to diagnose cancer, Multiple Sclerosis (MS) and other conditions Figures 1220 and 1221 show one of these machines and the image produced.

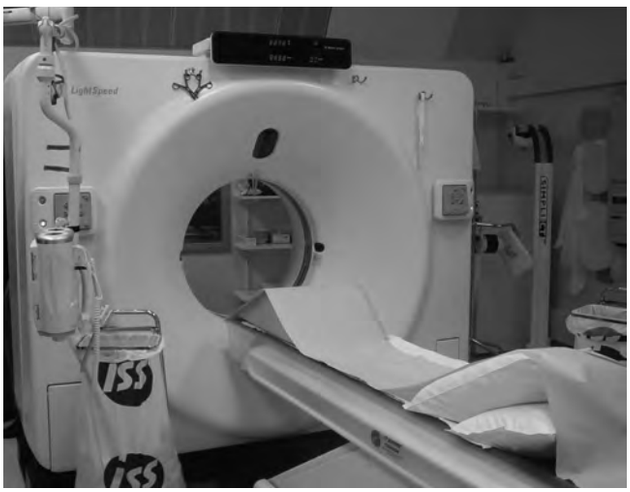


Figure 1220 A MRI body scanner

Figure 1221 The image produced by the scanner

A9 NMR SPECTROSCOPY [HL]

A.9.1 Explain the use of tetramethylsilane (TMS) as the reference standard.

It was seen above that when organic compounds are placed in an intense magnetic field, hydrogens in different 'chemical environments' absorb radio waves of slightly different frequencies. This 'chemical shift' (often given the symbol δ) is however very small – in the parts per million (ppm) range. The frequency of radiation is very dependent on the strength of the applied magnetic field and it is difficult to ensure that this remains constant to a comparable accuracy, which initially meant that it was difficult to obtain reproducible results. The problem is now overcome by mixing another substance with the sample and recording the frequency at which absorption occurs relative to this 'internal standard'. The substance chosen as the internal standard is **tetramethylsilane** (TMS) which has the formula $(CH_3)_4Si$. This has the advantage of being chemically inert, producing a single strong signal (as it has 12 hydrogens in identical chemical environments) and, because of the low electronegativity of silicon, it absorbs radiation of a frequency rather different from that of most other organic compounds. It does not interfere with their absorption signals. The chemical shift (in ppm) is then measured relative to this arbitrary standard being taken as 0 ppm.

A.9.2 Analyse ^{1}H NMR spectra.

One further complication is that the absorptions of hydrogen atoms on neighbouring carbon atoms interfere with each other and in a high resolution spectrum this leads to splitting of some absorptions into a number of closely grouped peaks. This is shown in Figure 1222 overleaf, for ethanol, in which some of the peaks are seen to split. The reason for this is that the precise frequency at which the absorption occurs is influenced by the direction of the magnetic field of any hydrogens attached to the neighbouring carbon atom. The signal of the CH_3– group in ethanol (δ = 1.1) is therefore affected by the alignment of the hydrogens of the $–CH_2–$ group. There are three combinations for this (↑↑, ↑↓ and ↓↑, or ↓↓) so the signal is split into three (a triplet) with an intensity ratio of 1:2:1

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(the same as the probabilities of the three states of the CH_2 hydrogens). Similarly the signal of the $-CH_2-$ group ($\delta = 3.8$) is split into a quartet by the possible alignments of the hydrogens in the CH_3- group (↑↑↑; ↑↑↓, ↑↓↑ & ↓↑↑; ↑↓↓, ↓↑↓ & ↓↓↑; ↓↓↓, hence a 1:3:3:1 ratio). The general rule is that the number of peaks is equal to the number of hydrogens on the neighbouring carbon plus one. The O–H signal is not split because the rapid exchange of this atom between ethanol molecules averages out the different possible spins.

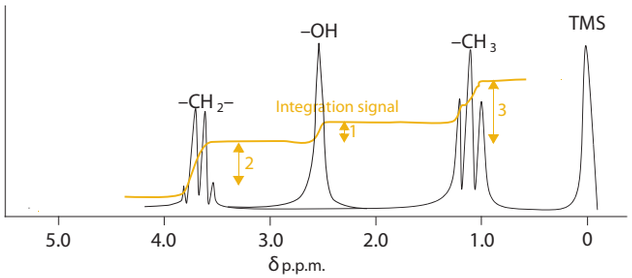

Figure 1222 High resolution NMR spectrum of ethanol with TMS reference

The strength of this technique is demonstrated by the very different spectra of the isomers of C_4H_9OH shown in Figure 1223 below.

You should be able to work out from the number of chemical environments and the ratio of hydrogen atoms in these, which spectrum belongs to which isomer [$(CH_3)_3COH$; $(CH_3)_2CHCH_2OH$; $CH_3CH(OH)CH_2CH_3$; $CH_3CH_2CH_2CH_2OH$]. The answer follows, can you also work out how the splitting of the peaks occurs?

(Answer (a) - butan-2-ol; (b) – 2-methylpropan-2-ol; (c) – butan-1-ol; (d) - 2-methylpropan-1-ol)

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Nucleons (protons and neutrons), like electrons have a property called **spin** which has a magnetic moment associated with it. In an external magnetic field they can either align themselves with or against the magnetic field. In many nuclei there is an even number of nucleons, which pair up (like electrons) and so their spins cancel each other out, so that the nucleus does not have an overall magnetic moment. Where there is an odd number of nucleons the nucleus has a net spin and magnetic moment. These have the same energy unless an external magnetic field is present in which case the magnetic field of the nucleus must either line up with the field (lower energy) or against the field (higher energy) – see Figure 1224a. The frequency corresponding to this small difference in energy ($\Delta E = hf$)

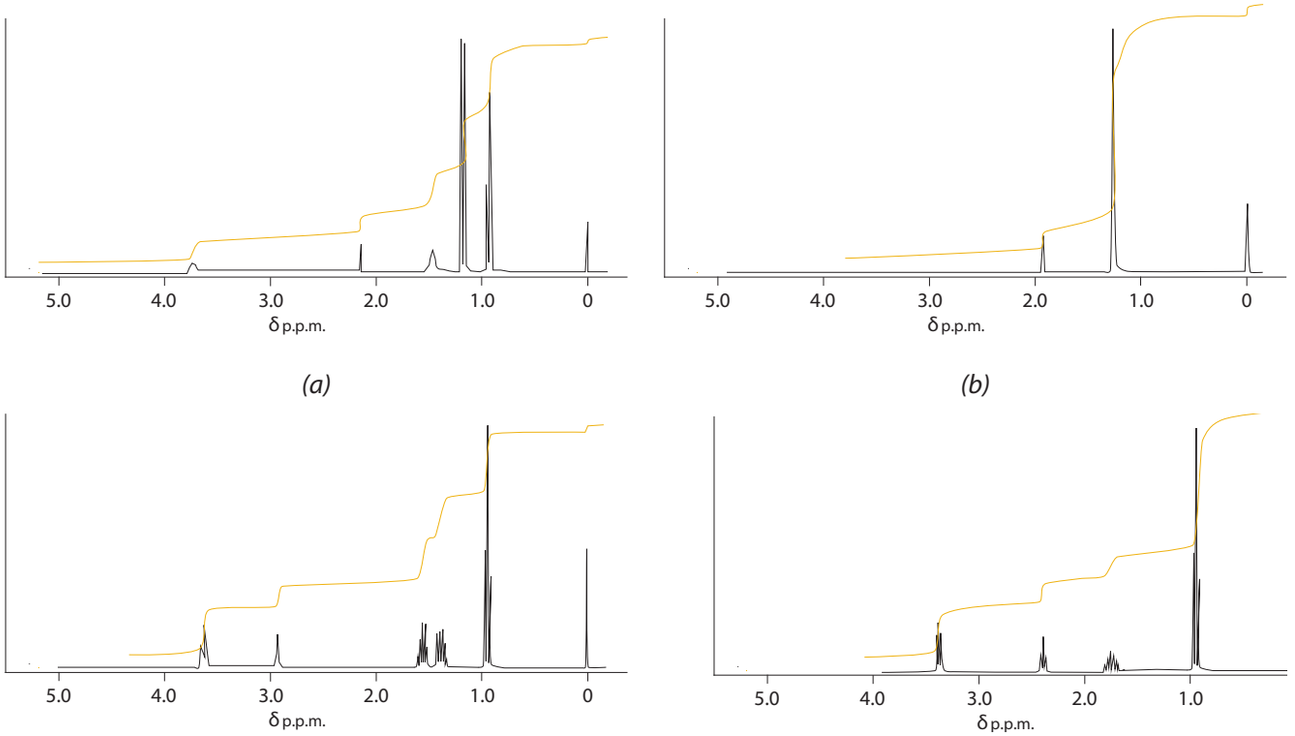

Figure 1223 (a), (b), (c) and (d) The NMR spectra of C_4H_9OH isomers

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falls in the radio waves frequency region. The nucleus can absorb radiation of just this frequency (in the radio frequency region) and the spin 'flips' to the higher energy orientation.

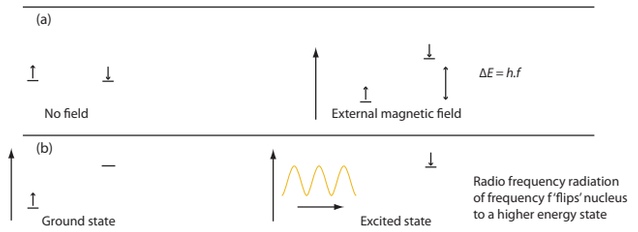


Figure 1224 The effect of a magnetic field on the spin states of a nucleus with residual spin and excitation by the absorption of radio frequency radiation

Useful nuclei with a non-zero magnetic moment are ^{1}H, ^{13}C, ^{19}F and ^{31}P. Such nuclei can therefore absorb radio frequency energy of the appropriate frequency and move ('flip') from the lower energy state to the higher energy state. An NMR spectrum is obtained by placing the sample in a cylindrical glass tube with a standard (TMS) in a strong magnetic field, often produced using superconducting magnets. The sample is surrounded by a radio frequency generator and receiver and the NMR spectrum is obtained by varying the strength of the magnetic field.

The reason for the chemical shift is that when electrons are in a magnetic field they orbit in such a way as to set up a magnetic field that opposes the applied field (**Lenz's law**). This means that the magnetic field experienced by the nucleus, and hence the precise frequency at which it absorbs radiation, depends on the electron density near to the nucleus and therefore on the chemical environment of the nucleus. For example, because chlorine is more electronegative than iodine, the hydrogen atom in H-Cl has less electrons near to it, so that it experiences a stronger magnetic field and will therefore absorb radiation of a higher frequency than the hydrogen atom in H-I. In molecules that have a number of hydrogens in different chemical environments, then each hydrogen will produce an absorption at a different frequency and the strength of the absorption will be proportional to the number of hydrogen atoms in that environment.

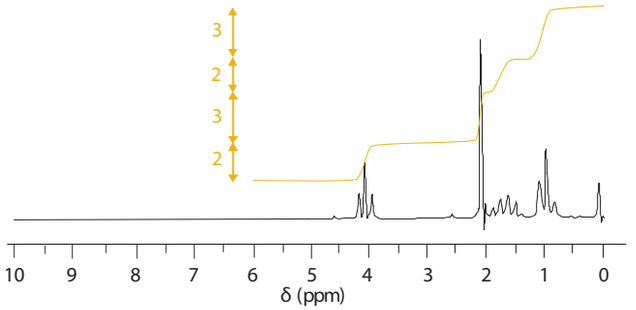
EXTENSION

Exercise

1. Use a table of chemical shifts to predict the absorptions that would occur (both the chemical shift in ppm and the relative intensity) in the NMR spectrum of 3–methylbutanal.

   There are a number of possible isomers of this compound. Name the isomer that would have the simplest NMR spectrum and describe the NMR spectrum that it would produce.

2. Identify the organic molecule responsible for the NMR spectrum shown below, explaining how you reach your conclusion, including reference to the splitting pattern.



3. Explain why some atomic nuclei, such as ^{19}F, give rise to NMR spectra whilst others, such as ^{16}O do not. Explain why the nuclei of a particular isotope do not always absorb energy of exactly the same frequency.

A6 ATOMIC ABSORPTION (AA) SPECTROSCOPY

A.6.1 State the uses of Atomic Absorption spectroscopy.

Atomic absorption spectroscopy is a technique that is widely used for the detection of metal ions in samples. The technique can be used to detect metals in a wide variety of samples, such as water, blood, soil and food samples, for example:

- Aluminium in blood serum
- Calcium in blood serum, plants, soil samples, in water (for water hardness)
- Copper in copper-based alloys
- Chromium in sea water
- Iron in plants

It can be highly sensitive with detection levels in the parts per million range, though recent developments of the technique (such as graphite furnace AA spectroscopy)

can operate at the parts per billion level. The technique also requires only very small samples, less than 1 drop of solution, to give reliable results. Another major strength of the technique is that it is not necessary to separate the metal from other components as it is highly selective and other components, such as other metal ions, do not interfere with it.

A.6.2 Describe the principles of atomic absorption.

The principles of atomic absorption are based on the fact that when metal atoms are excited by heat, the atoms absorb light. Each metal absorbs light of characteristic wavelength or frequency:

Metal	Zn	Fe	Cu	Ca	Na
Wavelength / nm	214	248	325	423	589

Figure 1226 Characteristic wavelengths absorbed by some metals

The non-excited vaporized metal atom absorbs its characteristic frequency from an external source and becomes excited, that is, it causes transition from the ground state to the excited state as shown in Figure 1227:

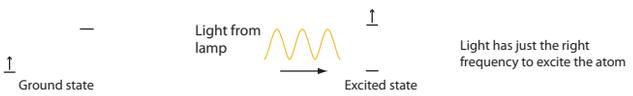


Figure 1227 Illustrating atomic absorption

The ratio of the intensity of the transmitted light to that of the incident light energy is proportional to the concentration of the metal atoms present. Thus, as the concentration goes up, the absorbance goes up, so that a calibration curve obtained by using standard solutions of known concentrations, can be used to determine the concentration of that metal in the unknown.

A.6.3 Describe the use of each of the following components of the Atomic Absorption spectrometer: fuel, atomizer, monochromatic light source, monochromatic detector, read-out.

Atomic absorption spectroscopy relies on the fact that metal atoms will absorb light of the same frequency as that emitted by excited atoms of that metal. For that reason if the intention is to analyse a sample for copper, then a lamp is used in which copper is excited so as to emit specific frequencies of light corresponding to the emission line spectrum of copper. Light from the lamp is then shone through a long, narrow, very hot flame (see Figure 1228, below), usually produced by burning a mixture of ethyne and oxygen, into which the sample is introduced. The light then passes through a monochromator, which acts like a prism to select just the frequency of light being used, then on to a sensitive detector. The detector measures the decrease in the intensity of that light which occurs when the sample is introduced into the flame. The technique relies on the fact that it is only atoms of that particular element that will absorb light of exactly the same frequency as that emitted by the lamp. If it is to be used for a different metal, then a different lamp containing that metal has to be used. Figure 1228 below shows a simple atomic absorption spectrophotometer:

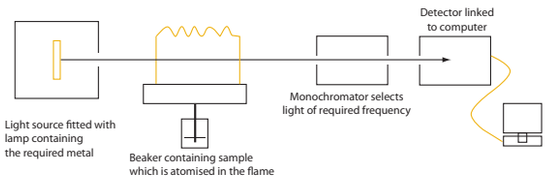


Figure 1228 A schematic diagram of an atomic absorption spectrometer

The sample is atomised into the flame. The simplest way of doing this is to atomise microscopic droplets of a solution into the gas supply to the flame. The heat of the flame firstly evaporates the solvent, then it causes the compound to split into individual atoms which absorb the light. The amount of light absorbed is proportional to the concentration of atoms of the metal in the flame and hence in the sample.

A.6.4 Determine the concentration of a solution from a calibration curve.

Atomic absorption spectroscopy can be used both for qualitative analysis, for example to detect the presence of lead in a gunshot residue, as well for as quantitative analysis to determine how much of the metal is present in the sample. The intensity of the absorption varies a little bit with conditions, such as the temperature of the flame and so therefore the instrument is usually calibrated using solutions of known concentration of the metal that is being detected. Using these data, a calibration curve can be drawn which may be used to find the concentration of the unknown. For example consider these data for

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the analysis of a sample of waste water for chromium at 358 nm. Figure 1229 illustrates how (a) data and (b) a calibration curve can be used to find the concentration of an unknown.

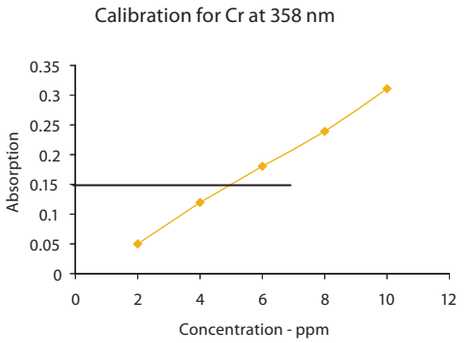

Figure 1229 (a) and (b)

It can be seen from Figure 1229 that the concentration of chromium in the unknown is approximately 5 ppm, though analysis of the data and calibration samples by a computer would yield a more precise result.

Exercise A6

1. Which of the following would be best detected using atomic absorption spectroscopy?

   A The presence of steroids in a urine sample from an athlete.
   B The concentration of mercury in the effluent from a brine electrolysis plant.
   C The presence of toxic carbon monoxide in gases from a mine.
   D The concentration of vitamin C in a sample of orange juice.

2. A 0.6230 g sample containing a compound of sodium is dissolved in water and diluted to 100 cm^3 in a volumetric flask. It is analysed in an atomic absorption spectrometer using the 589 nm sodium line and shows an absorbance of 0.30. The following additional data was also obtained:

[Na] / mg dm^3	0.00	0.50	1.00	1.50	2.00	2.50
Absorbance	0.00	0.10	0.26	0.36	0.46	0.62

   Draw a calibration curve in order to obtain the concentration in mg dm^3 and percentage of sodium in the sample.

3 Lead is extracted from a sample of blood and analyzed at a 283 nm and gave an absorbance of 0.340 in an atomic absorption spectrometer. The following additional data was also obtained by the subsequent dilution of a standard solution of lead ions:

$[Pb^{2+}]$ / ppm	0.000	0.100	0.200	0.300	0.400	0.500
Absorbance	0.00	0.116	0.216	0.310	0.425	0.520

Draw a calibration curve and determine the lead content of blood in ppm.

A7 CHROMATOGRAPHY

A7.1 State the reasons for using chromatography.

In chemistry, the concept of a pure substance (that is one that contains only one compound) is vital and so techniques that can identify whether a sample is a pure substance or a mixture, and separate mixtures of substances into their pure components are important. Most of these techniques are used for analytical purposes, that is to see what is present in the mixture (for example to see if a particular amino acid is present in the mixture from the hydrolysis of a protein), rather than to obtain a pure sample of one of the components of the mixture (for example to produce a sample of chlorophyll from the extract of a plant leaf).

Sometimes **chromatography**, as its name suggests, is used to separate coloured substances and the components can then be detected by their colour. More often it is used with colourless substances and in these cases a variety of special techniques must be used to detect the presence of the components of the mixture. These include the use of UV light if a fluorescent component is present, or a solution that makes a component visible by reacting with it, such as using ninhydrin solution to identify the presence of amino acids.

A7.2 Explain that all chromatographic techniques involve adsorption on a stationary phase and partition between a stationary and a mobile phase.

The term chromatography is used to describe a range of closely related techniques used to separate mixtures that involve the interaction of the components with two phases; a **stationary phase** that does not move and a **mobile phase** that moves through the stationary phase. The components are separated according to how much time the components

spend in the different phases, as a result of the differences in their attractions for the stationary and mobile phases. Imagine a moving conveyor belt and a group of people standing together alongside it. The people all jump on to the conveyor belt, but one group (A) counts to five and then jumps off. When off, they count to ten and jump on again, then after five, off again and so on. The second group (B) count to ten when they jump on the conveyor and five when they jump off, ten when they are on again. After a minute or so, it is quite obvious that the people in group B will have travelled rather further than those in group A, so that the 'mixture' of people has been separated.

All chromatography depends on the relative tendencies of the different components to bond to the surface of the stationary phase, or to remain in the mobile phase. A component that bonds strongly to the stationary phase will not move very far, whereas one that bonds more strongly to the mobile phase will move faster. The separation between the stationary and mobile phases operates on the principle of partition or adsorption. Partition involves the way in which components of a mixture distribute themselves between two immiscible liquid phases, depending on their solubility in each phase, whilst adsorption involves the way a substance bonds to the surface of a solid stationary phase. The simplest chromatographic techniques are paper chromatography; thin–layer chromatography (TLC) and column chromatography. The way in which these are carried out, and the underlying theory of each, is described below.

A7.3 Outline the use of paper chromatography, thin-layer chromatography (TLC) and column chromatography:

PAPER CHROMATOGRAPHY

In **paper chromatography** a spot of the mixture is applied to absorbent paper, rather like filter paper. The end of this is then dipped in the solvent used to 'develop' the chromatogram. The solvent soaks up the paper by capillary action, moving past the spot where the mixture was applied and onwards. The components that bond strongly to the solvent will be carried along in the direction that the solvent is moving, whereas those that do not bond to it will remain almost stationary. Figure 1232 below shows one common arrangement for carrying out paper chromatography, though many others are possible.

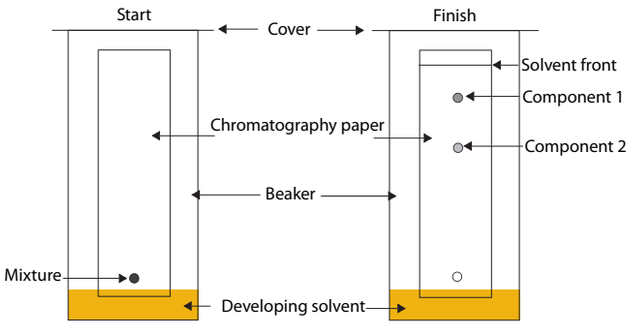

Figure 1232 Paper chromatography

In the above diagram, as the organic solvent soaks through the paper, Component 1 is very soluble in the solvent, which is the mobile phase, and only poorly in water held in the absorbent pores of the paper, which acts as the stationary phase. Component 1 has therefore moved almost as far as the solvent. Component 2, because of its different structure, bonds more strongly to the stationary aqueous phase and so does not move as far. This technique relies on the partition of the components of the mixture between a mobile non–aqueous phase and a stationary aqueous phase. Paper chromatography can be used to separate the coloured components of an ink, or the different amino acids from a mixture of amino acids.

THIN LAYER CHROMATOGRAPHY

Thin layer chromatography (TLC) is very similar in practice to paper chromatography. The physical arrangement being almost identical to that shown in the previous diagram. The difference is that the stationary phase is a thin layer, usually of silica (silicon dioxide, SiO_2) or alumina (aluminum oxide, Al_2O_3), on a glass or plastic support. This means that the separation is not the result of the partition of the components between two liquids, but it depends on the extent to which they bond to the surface of (i.e. are adsorbed by) the stationary oxide layer, which in turn mainly depends on the polarity of the substance. TLC is used to separate similar mixtures to paper chromatography, but because the particles in TLC are much finer than the pores in paper, it usually gives better separation.

In both paper and thin layer chromatography, components can be identified by their R_f value, where:

$$R_f = \frac{\text{Distance moved by component}}{\text{Distance moved by solvent}}$$

In the example in Figure 1232, the R_f value of component 1 is greater than that of component 2.

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Example

Find the R_f value of the component shown on the paper chromatogram below:

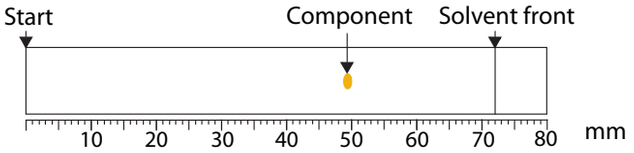


Figure 1233

Solution

$$R_f = \frac{\text{Distance moved by component}}{\text{Distance moved by solvent}} = \frac{49}{72} \approx 0.68$$

COLUMN CHROMATOGRAPHY

The principle of **column chromatography** is very similar to thin layer chromatography, as the stationary phase is usually silica or alumina and separation depends on whether a component is strongly adsorbed onto the surface of this or remains dissolved in the mobile phase of solvent used to elute the column. The oxide powder is packed into a column with the solvent and the mixture applied at the top of the packing, as shown in Figure 1234. The solvent (also known as the **eluant**) is allowed to slowly drip out of the bottom of the column, controlled by a tap, and fresh solvent added at the top so that the packing never becomes dry. As the mixture moves down it will separate out into its components, as shown. This technique can be used to obtain a pure sample of the various components as they can be collected separately when they elute from the bottom of the column and the solvent evaporated. If the components are colourless, then separate fractions of the eluate (the solution leaving the column) must be collected and tested for the presence of the components of the mixture.

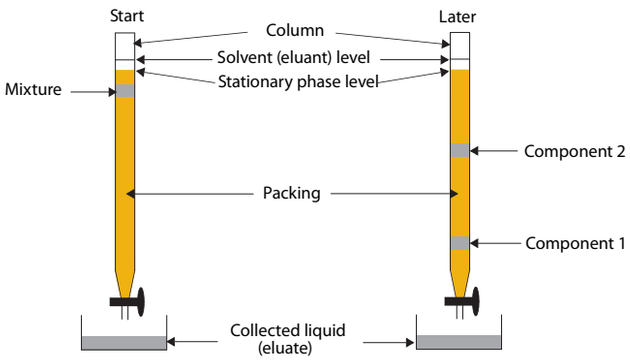


Figure 1234 An illustration of column chromatography

A10 CHROMATOGRAPHY [HL]

A.10.1 Describe the techniques of gas-liquid chromatography (GLC) and high-performance liquid chromatography (HPLC).

GAS LIQUID CHROMATOGRAPHY

In **gas liquid chromatography** (GLC), the mobile phase is a gas and the stationary phase is packed into a very long (often a number of metres) thin column, that is coiled into a helix, see Figure 1235. There are various types of stationary phases that may be used. Sometimes the column is packed with an oxide (usually SiO_2 or Al_2O_3), or more frequently these days (HRGC - high resolution gas chromatography) a very thin column is coated on the inside with an oxide layer, and in these cases separation occurs because molecules of the mixture are adsorbed onto the surface of the oxide. Sometimes, the oxide will just be acting as a support for a high boiling point oil or wax. In this case separation depends on the partition of the components between the gas phase and solution in the oil.

OPTION

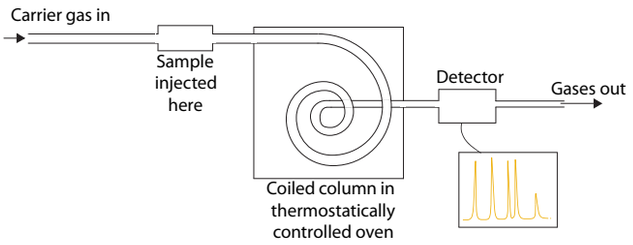
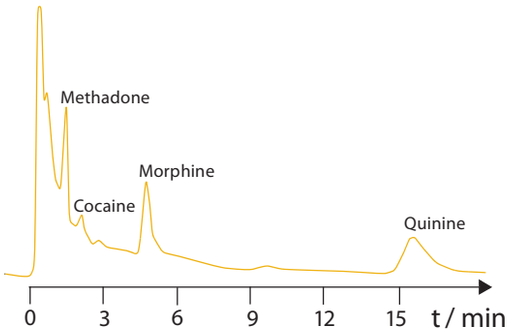


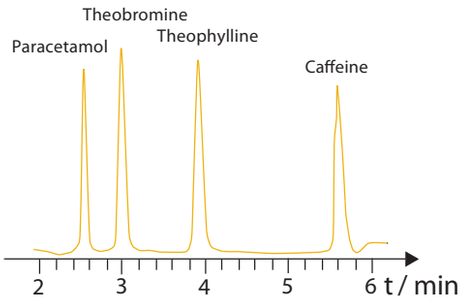
Figure 1235 Schematic diagram of gas chromatography

The mixture, which must vaporise at the temperature used, is usually injected, by means of a hypodermic syringe, into a steady gas flow at the start of the column. One great advantage of the technique is the very small samples required, of the order of a microlitre (1 μl = 10^{-6} dm^3). The rate at which the sample passes through the column can be controlled by the temperature and for this reason the column is housed in an oven. It is therefore important that samples used in GLC are thermally stable.

The components of the mixture are detected as they reach the end of the column, either by the effect they have on the thermal conductivity of the gas, or by the current that results from the ions formed when they are burnt in a flame. The results are shown as a graph of the detector signal against the time since the mixture was injected into the gas flow. The components can often be identified from the time taken for them to emerge from the column (called the **retention time**) and the area under the peak is proportional to the amount of the component in the mixture. Typical GLC chromatograms are shown in Figure 1236(a) and (b).



(a) typical glc to show the detection of drugs in a urine sample



(b) typical hplc to show the components of a proprietary cold treatment

Figure 1236 Typical glc and hplc chromatograms

It can be seen that gas chromatography allows the number of components in a mixture to be identified and the relative amounts of these can be determined from the area under the peak. For more precise work, the system can be calibrated using samples of known concentration under identical conditions.

GLC is used to identify components that can vaporize without decomposition such as analysis of vegetable oil mixtures, analysis of gas mixtures from underground mines or from petrochemical works, analysis of components of fruit odours, detection of steroids or drugs in urine samples from athletes, and blood alcohol levels.

A very powerful technique **Gas Chromatography – Mass Spectrometry**, (GC–MS) involves coupling the output of the gas chromatography column to the input of the mass spectrometer. This means that each component is definitely identified as it elutes by means of its mass spectrum. This is particularly useful in food and drug testing as well as in forensic science.

OPTION

Technique	Stationary Phase	Mobile Phase	Typical application
Paper chromatography	Trapped water in the paper	Organic solvent	Detection of amino acids in a mixture Testing food colours to see if they are single dyes or mixtures
Thin layer chromatography	Oxide coating	Organic solvent	Detection of amino acids in a mixture Testing food colours to see if they are single dyes or mixtures
Column chromatography	Oxide packing or ion exchange resin	Organic solvent	Preparative, e.g. separation of the chlorophylls and carotene in plant extract
Gas-liquid chromatography	Oxide or non-volatile liquid on the solid support	Gas	Analysis of vegetable oil mixtures Analysis of gas mixtures, especially from mines Analysis of components of fruit odours Detection of levels of alcohol in blood Detection of drugs in urine Detection of steroids
High Performance Liquid Chromatography	Oxide packing	Liquid	Analysis of sugars in fruit juices Analysis of additives in margarine Analysis of pesticide & herbicide residues Oil pollutants Alcohol in drinks

Figure 1237 Summary of some chromatographic techniques

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The principle of **High Performance Liquid Chromatography** (HPLC) is basically very similar to gas chromatography except that the mobile phase is a liquid, forced under high pressure (up to 10^7 Pa) through a rather shorter column (usually 10–30 centimetres long), rather than a gas. Its advantage over gas chromatography lies in the fact that it can be used for non-volatile and ionic substances, as well as those that are not thermally stable enough for GLC. One of the major weaknesses of this technique is that the detector systems are less sensitive than those usually used in gas chromatography. The most commonly used detection system is the absorption of UV light, though there are a wide variety of other detector systems (e.g. fluorescence and conductivity) that find specialist use.

Usually (called 'normal phase') the packing is either a polar oxide (silicon dioxide or aluminium oxide), or an inert support coated with a thin layer of a polar liquid which acts as the stationary phase and a non–polar liquid is used as the mobile phase. In such a system, the less polar components elute before the more polar. The polarity of the phases can however be reversed ('reverse phase', i.e. a non-polar packing and a polar mobile phase) so that the more polar components elute first. A typical HPLC chromatogram is shown in Figure 1236(b).

HPLC is used for temperature-sensitive components and for chemicals that do not vaporize easily because of high boiling points or ease of decomposition, for example, in the analysis of oil pollutants in soil and water samples, analysis of antioxidants, sugars and vitamins in fruit juices and analysis of drugs in blood and urine.

A.10.2 Deduce which chromatographic technique is most appropriate for separating the components in a particular mixture

When facing an analytical problem it is important to select the correct technique. This will depend on what is required. Is it qualitative or quantitative? Are the components known or must they be identified? How much of the material is available (kilograms or micrograms?). How low are the concentrations of the substances to be detected (~0.01 mol dm^{-3} or 10^{-6} mol dm^{-3}). The choice

OPTION

also depends on the nature of the sample. Is it volatile? How polar is it? Is it thermally stable?

- For a stable, volatile sample, generally gas chromatography on a suitable column will offer the best solution.
- For a non–volatile or thermally unstable sample HPLC will often provide the solution.
- Column chromatography is most suitable for preparative purposes, whilst paper and thin layer techniques involve the minimum amount of apparatus, if all that is required is a simple qualitative check.

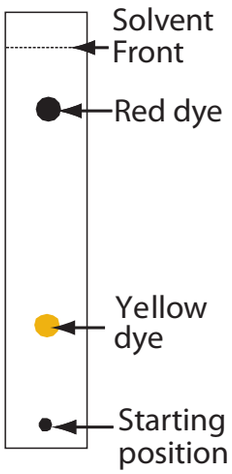
Some common applications of the different techniques are given in Figure 1237.

Exercise A10

1. Many chromatographic techniques are used to detect the presence of a particular substance. Column chromatography can however also be used as a preparative technique, i.e. to produce a sample of a substance. Explain how you would attempt to use this technique to produce pure samples of the different dyes comprising universal indicator.

2. (a) Describe the technique of paper chromatography to separate a mixture of dyes and explain the chemical principle behind it.
   (b) Define the term R_f. Describe how it could be determined for a component of a mixture and explain the significance of an R_f value of 0.0 and one of 1.0
   (c) Describe how you would detect the presence of an illegal substance added to a product with the food dye.

3. a) All chromatography depends upon a separation between two phases. What are these phases in the case of paper chromatography?



The paper chromatogram of an orange dye is illustrated. Explain the separation of the two components in terms of their relative affinities for the stationary and mobile phases.

b) Calculate the R_f values of the components.

c) Gas–liquid chromatography (GLC) and high performance liquid chromatography (HPLC) are both far more widely used than paper chromatography. Give at least two reasons why these techniques are preferred.

d) Give an example of a mixture for which GLC would give better results than HPLC and one for which the reverse is true. In each case state why that technique is to be preferred.

4. When a mixture of the four isomeric alcohols with the molecular formula C_4H_9OH is passed through a gas chromatography column they are separated with 2-methylpropan-2-ol eluting first and butan-1-ol eluting last.

a) What does this show about the relative attraction of butan-1-ol and 2-methylpropan-2-ol for the packing of the column? Explain how this leads to the separation of the two compounds.

b) How could the relative amounts of the four isomers in the mixture be found?

c) By what methods might the alkanols be detected as they elute from the column?

d) How would the time taken for the substances to elute be affected if the temperature of the column was increased?

OPTION

A8 VISIBLE AND ULTRAVIOLET (UV-VIS) SPECTROSCOPY [HL]

Ultraviolet-visible spectroscopy, a very similar technique to infrared spectroscopy, but it uses UV and visible light instead of infrared, in the assaying of metal ions, organic structure determination and the detection of drug metabolites.

A.8.1 Describe the effect of different ligands on the splitting of the d orbitals in transition metal complexes

A.8.2 Describe the factors that affect the colour of transition metal complexes

The energy carried by a quantum of light in the UV (wavelengths between ~250 – 400 nm) and visible regions (wavelengths between ~400-700 nm) of the spectrum corresponds to the difference in energy between filled and unfilled electron orbitals in many ions and molecules. In many ions, for example sodium ions, the difference in energy between the highest filled orbital and the lowest unfilled orbital (i.e. the 2p and the 3s for Na^+) is quite large and so they only absorb very short wavelength UV light, hence their ions are colourless. In the transition metals, the difference in energy between filled and unfilled split d-orbitals is much smaller so that these ions in solution absorb energy in the far UV and visible regions, the latter being responsible for the fact that many of these ions are coloured. For example aqueous copper(II) ions appear blue in colour because they absorb light in the red and green regions of the visible spectrum; the colour observed being the complementary colour of the colours absorbed.

In transition metals, light can be absorbed because, even though in an isolated atom the d-orbitals are all of the same energy, when the atom is surrounded by charged or polar ligands the interaction of the different orbitals with the electric fields of these ligands varies and hence they have different energies. This usually causes the d-orbitals to split into two groups with three orbitals at a lower energy and two at a slightly higher energy.

The difference in energy between these two groups of orbitals is smaller than that between most electron orbitals and corresponds ($\Delta E = hf$) to light in the visible region of the spectrum. The exact difference in energy between the two groups of d-orbitals, and hence the colour of the light absorbed (remember the colour it appears is the complementary colour of the absorbed light) depends on a number of factors:

- the element being considered (especially the nuclear charge)
- the charge on the ion
- the ligands surrounding the ion
- the number and geometrical arrangement of the ligands.

The first point is easily illustrated by considering manganese(II), which has an almost colourless hexaaquo ion $[Mn(H_2O)_6]^{2+}$ and iron(III), which has a yellow-brown hexaaquo ion $[Fe(H_2O)_6]^{3+}$, even though they both have the same electronic structure [Ar] $3d^5$. This is a result of the differing charges on the two nuclei and the effect of this on the electron orbitals. An example of the second factor is the two oxidation states of iron, which have differing numbers of d-electrons and as a result $[Fe(H_2O)_6]^{2+}$ is pale green and $[Fe(H_2O)_6]^{3+}$ is yellow-brown. Here the nuclear charge is constant, but there is a difference in the electronic structures - [Ar] $3d^6$ and [Ar] $3d^5$ respectively.

Changing the identity of the ligand changes the degree of splitting depending on the electron density of the ligand and the extent to which it repels the electrons in the d-orbitals. The nature of the ligand is well illustrated by copper(II). With water there is the familiar pale blue colour of the hexaaqua ion, $[Cu(H_2O)_6]^{2+}$. If the ligands are gradually replaced by ammonia, to give for example $[Cu(NH_3)_4(H_2O)_2]^{2+}$, the colour deepens to a dark royal blue as the energy gap between the d-orbitals is increased. If the water ligands are gradually replaced by chloride ions the colour changes through green to the yellow of $[CuCl_4]^{2-}$ because chloride ions cause less splitting of the d-orbitals.

Obviously this last case also results from a change in the number and geometry of the ligands, which can lead to quite marked changes of colour. The hexaaqua ion of cobalt, $[Co(H_2O)_6]^{2+}$ is, for example, pale pink (i.e. blue, green and yellow light are weakly absorbed), but the tetrachloro ion, $[CoCl_4]^{2-}$ is dark blue (i.e green, yellow and red light are strongly absorbed).

OPTION

A.8.3 State that organic molecules containing a double bond absorb radiation.

A.8.4 Describe the effect of the conjugation of double bonds in organic molecules on the wavelength of the absorbed light.

A.8.5 Predict whether or not a particular molecule will absorb ultraviolet or visible radiation.

In the same way that atoms and ions have atomic orbitals, molecules have molecular orbitals, some of which are filled, others are unfilled. In simple molecules, such as water, as in simple ions, the difference in energy between the highest filled orbital and the lowest unfilled orbital is again quite large and so they too only absorb very short wavelength UV light and hence appear colourless. The difference in energy in molecules that have double bonds (i.e. C=C and C=O, structural elements known as '**chromophores**') is much less, especially if these are 'conjugated' (that is there are alternate double and single bonds), and/or involve extensive delocalised bonds (such as in a benzene ring). Molecules of this kind absorb light in the far UV and visible regions. For example 1,10–diphenyl–1,3,5,7,9–decapentene which, as shown below, has two benzene rings and an extensive chain of conjugated double bonds, is an orange colour because it absorbs blue and green light.

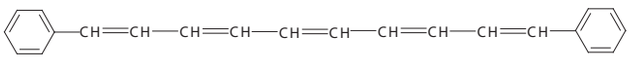
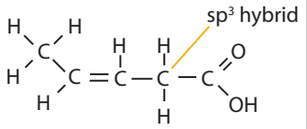
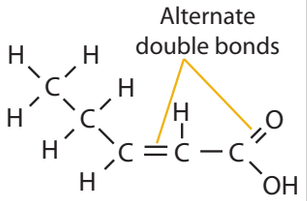
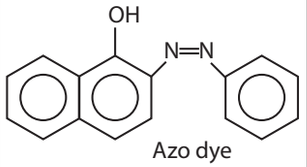


Figure 1239 The structure of 1,10-diphenyl-1,3,5,7,9-decapentene

If we examine the structures of a number of compounds to identify these structural elements, it is possible to predict to what extent these will absorb UV and visible light. Consider the following compounds:

2.3-dichloropentane contains only σ bonds and so will only absorb short wavelength UV light.	$CH_3CHClCHClCH_2CH_3$
Pent-3-enoic acid contains isolated double bonds (not alternate single and double bonds), hence no delocalization, so that it will only absorb in the mid UV region.	
In pent-2-enoic acid the single and double bonds are alternate, hence the double bonds are conjugated, so the UV absorption will be at a longer wavelength.	
It is only when there is an extended system of delocalised bonds and conjugated double bonds, as in the diazonium compound the absorption moves into the visible region to produce a coloured compound (in this case, red).	

Note the difference in the structures of phenolphthalein given below. In acid solution, species I (Figure 1243), it is colourless as there is limited delocalisation due to the presence of the sp^3 hybridised carbon. In alkaline solution, species II, there is extensive delocalisation possible due to the presence of the sp^2 hybridised carbon, and it is coloured pink.

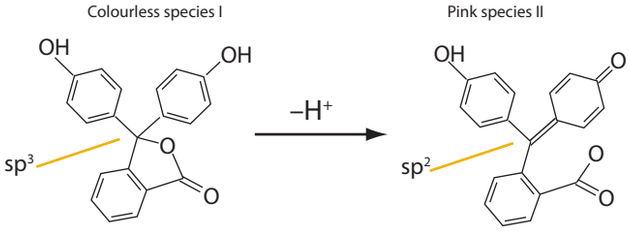


Figure 1243 The structures of phenolphthalein in acid and alkaline solution respectively

OPTION

Other substances that absorb visible light as a result of extended conjugated systems are (a) retinol (vital for vision) and (b) chlorophyll (vital for photosynthesis), the structures of which are shown in Figure 1244:

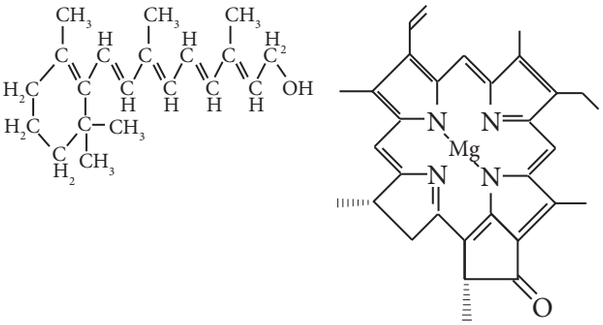


Figure 1244 (a) and (b) Some organic compounds that are coloured because they absorb visible light

Many drugs and their metabolites absorb light in the UV region so that UV/Vis spectrophotometry can also be used to detect and measure their concentrations. One other group of compounds for which the absorption of UV light is important is sun creams or sun blocks. Exposure to UV light damages the surface of the skin. If this occurs gently and in moderation then it results in the production of more melanin, a natural pigment, and a darkening of the skin – tanning. With excess exposure the damage to the skin can be severe (sun burn) and perhaps more seriously increases the risk of melanoma and other forms of skin cancer. To prevent this, sun blocks should be applied to the skin before long exposure to sunlight. These are compounds that strongly absorb UV light and as might be expected they have systems of extended conjugation. A typical example would be para-aminobenzoic acid (PABA) which is used as a 'sun-block' as shown in Figure 1245.

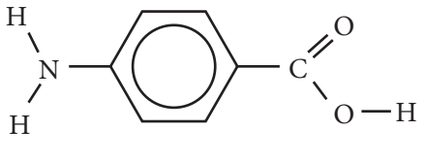


Figure 1245 The structure of para-aminobenzoic acid (PABA)

A.8.6 Determine the concentration of a solution from a calibration curve using the Beer–Lambert law.

The amount of light of a particular frequency which a solution absorbs will depend on the nature of the compound (which determines the molar extinction, or absorption, coefficient; ε), its concentration (*c* in mol dm^{-3}) and the distance the light passes through the solution (*l*, in cm). The intensity of the light (*I*) is found to decay exponentially as it passes through the solution, giving rise to a logarithmic relationship:

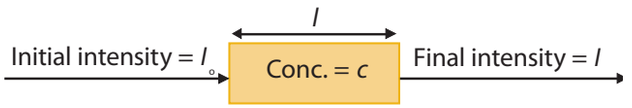


Figure 1246 Intensity of light decays exponentially

$$I = I_0 10^{-\varepsilon cl}$$

Taking logarithms:

$$A = \log_{10}\left(\frac{I_0}{I}\right) = \varepsilon cl$$

This is known as the **Beer–Lambert law**, and *A* is known as the absorbance of the solution (note that because it is a logarithm, absorbance (A) does not have units.), the reading given directly by most UV/visible spectrophotometers, though some also give the percentage transmittance, *T*, where:

$$T = \frac{I_0}{I} \times 100$$

The molar extinction coefficient (ε) is a measure of how strongly the compound absorbs light. The larger the value of ε, the stronger the absorption – the permanganate ion (MnO_4^-) for example has a very large extinction coefficient. This relationship means that a graph of absorbance (*A*) against concentration (*c*) is linear, making it easy to use this technique to determine the concentration of a given species in a solution. The molar extinction coefficient (ε) may be found from the gradient, knowing the path length (*l*). It is equal to the logarithm of the fractional decrease in intensity of the monochromatic light as it passes through 1 cm of 1 mol dm^{-3} solution and therefore has units of dm^3 mol^{-1} cm^{-1}.

OPTION

In fact the Beer-Lambert law only holds precisely for dilute solutions, so it is always best to produce a calibration curve, using solutions of known concentration, when using UV/Visible absorbance quantitatively. An example is the absorption of iodine in aqueous 0.10 mol dm^{-3} potassium iodide at 306 nm. The graph of absorbance against concentration as light passes through a 1 cm cell is shown in Figure 1247. As expected it is linear and from the gradient, the molar extinction coefficient may be determined as

$$\frac{2.3}{1 \times 0.015} = 153 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$$

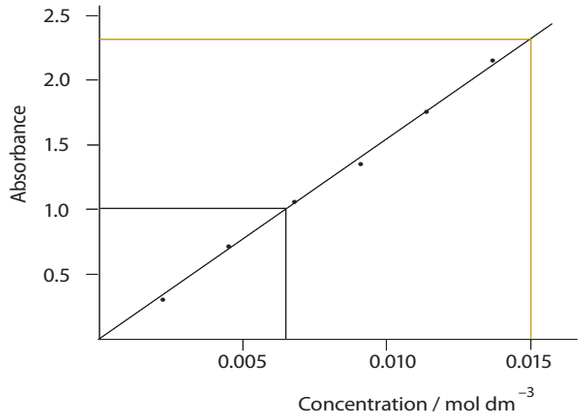


Figure 1247 The dependence of the absorption of iodine on concentration at 306 nm

If an unknown solution of iodine in 0.10 mol dm^{-3} potassium iodide had an absorbance of 1.0 at 306 nm its concentration can be read from the graph as 0.007 mol dm^{-3}.

Exercise A8

1. A 1×10^{-4} mol dm^{-3} solution of an organic compound in a 1 cm cell has an absorbance of 0.5 at a wavelength of 300 nm. What is the numerical value of its molar extinction coefficient at this wavelength.

   A 5×10^{-5}
   B 5×10^{3}
   C 2×10^{5}
   D 6.00×10^{-2}

2. At a particular wavelength the molar extinction coefficient of aqueous copper sulfate is 300 dm^3 mol^{-1} cm^{-1}. Approximately what percentage of the incident light of this wavelength will pass through a 0.010 mol dm^{-3} solution in a 1 cm cell?

   A 10%
   B 1%
   C 0.1%
   D 0.01%

3. (a) Transition metal ions tend to absorb light in the visible region of the spectrum. Explain why this occurs and why the precise colour may vary with the other species present.
   (b) In terms of the colours of light absorbed in this way, explain why aqueous nickel sulfate appears a green colour.
   (c) The intensity of green light passing through a particular sample of aqueous nickel sulfate is I_1. If the concentration of the salt is doubled, but all other factors are kept constant, how will the intensity of the light that now passes, I_2, be related to I_1? What changes must be made to the distance that the light passes through the solution in order to restore the intensity of the transmitted light to I_1?
   (d) Aqueous nickel(II) ions form a brightly coloured complex with an organic ligand. Various volumes of equimolar solutions of the two species are mixed and the absorbance recorded. Use the results below to derive the probable formula of the complex ion formed, explaining your method.

Volume of Ni^{2+} solution cm^3	Volume of ligand solution cm^3	Absorbance
0	10	0
2	8	1.27
4	6	1.71
6	4	1.26
8	2	0.63
10	0	0

OPTION

4. Use the IB Data Booklet to find the structural fomula of a coloured organic compound and describe the features of its structure that result in it absorbing visible light.