2.4 Experimental Determination of Structure

Elemental Microanalysis

Elemental analysis (also known as combustion analysis) can be used to determine the masses of the elements in a sample of an organic compound

- masses of carbon, hydrogen, sulphur and nitrogen can be determined
- empirical formula can be calculated

A known mass of a compound (~2mg) is burned in excess oxygen at a high temperature.

- Carbon → carbon dioxide CO_2
- Hydrogen → water H_2O
- Sulphur → sulphur dioxide SO_2
- Nitrogen → oxides of nitrogen NO_x

Mixture of gases carried by helium gas through a chromatography column to a detector

- Detector sends data to a computer which compares the length of time taken to travel through the chromatography column with known standards
- Computer calculates the masses of CO_2, H_2O, SO_2 and NO_x produced by combustion of the original compound and translates this into masses of the elements C, H, S and N in the original compound.
- Empirical formula can then be calculated

This technique cannot be used to show the presence of any other elements present e.g. oxygen

- Other techniques must be employed to calculate any other elements

Questions

1. A sample of an organic compound with a mass of 1.224g was completely burned in oxygen and found to produce 2.340g of CO_2 and 1.433g of water only.
   Calculate the empirical formula of the organic compound.

2. Oxalic acid is found in rhubarb and contains only the elements carbon, hydrogen and oxygen. When 1.540g of oxalic acid was burned in oxygen, 1.504g of CO_2 and 0.310g of water were formed. Calculate the empirical formula for oxalic acid.
   If the molecular mass of oxalic acid is 90.0, what is its molecular formula?

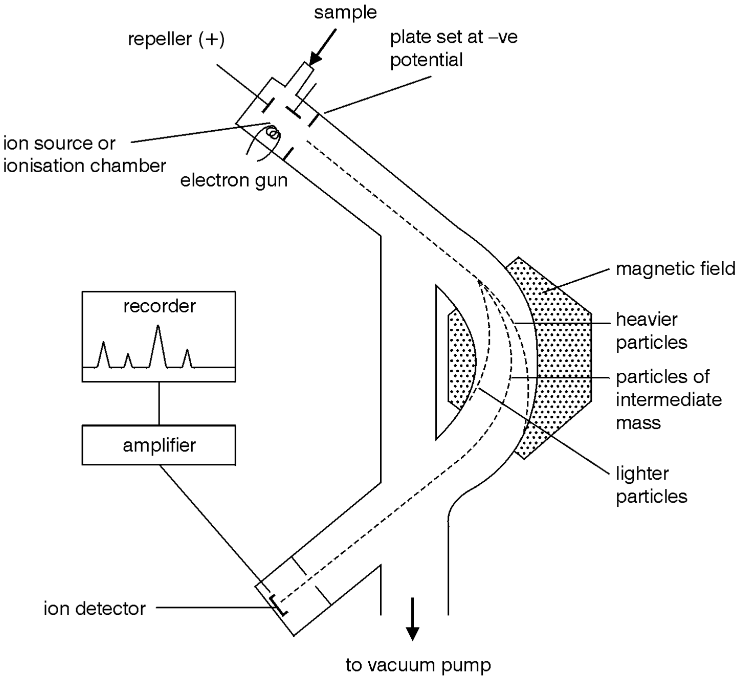
3. An organometallic compound known as ferrocene contains only the elements Fe, C and H. When 1.672g of ferrocene was combusted in oxygen, 3.962g of CO_2 and 0.810g of water were formed.
   Calculate the empirical formula of ferrocene.

Mass Spectroscopy

Mass spectroscopy is used to determine the accurate molecular mass and the structural features of an organic compound.

Mass Spectrometers work as follows:

1. Vacuum pump switched on to reduce pressure
2. Sample inserted and vapourised
3. Vapourised sample bombarded by electron beam in an ionisation chamber. Molecules become positively-charged as outer electrons are knocked off
4. Molecules can fragment as energy is available to break bonds
5. Parent ion and ion fragments are accelerated by an electric field into an ion beam
6. Beam of ions deflected by a magnetic field according to their mass/charge (m/z) ratio.
7. Mass Spectra produced by computer
   NB. Doubly charged ions can be produced and have a half-value m/z value as they have a double charge z (doubly charged ions are deflected more in the magnetic field)



In the mass spectrum formed, the peak with the highest mass is usually the unfragmented parent molecule

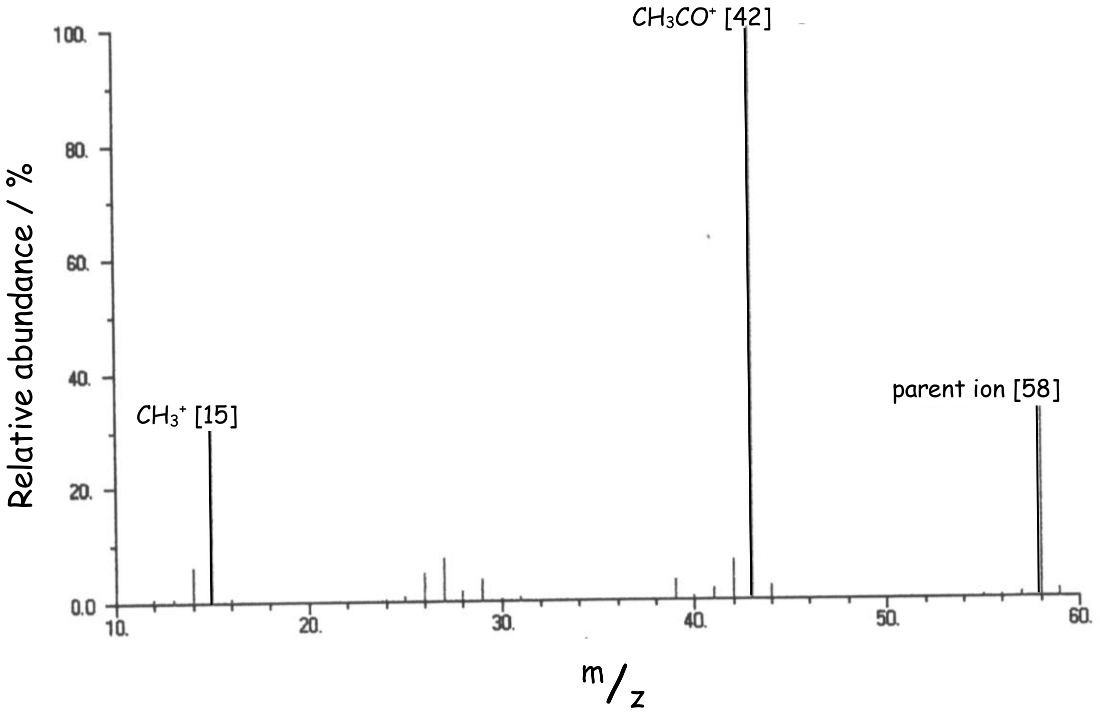
- Formula mass can be determined from this peak
- Fragments produced can help deduce the structure of the parent compound
- Computer data base of known samples mass spectra can reveal the identity of an unknown compound

Very accurate mass spectroscopy can reveal the identity of organic compounds of similar molecular mass

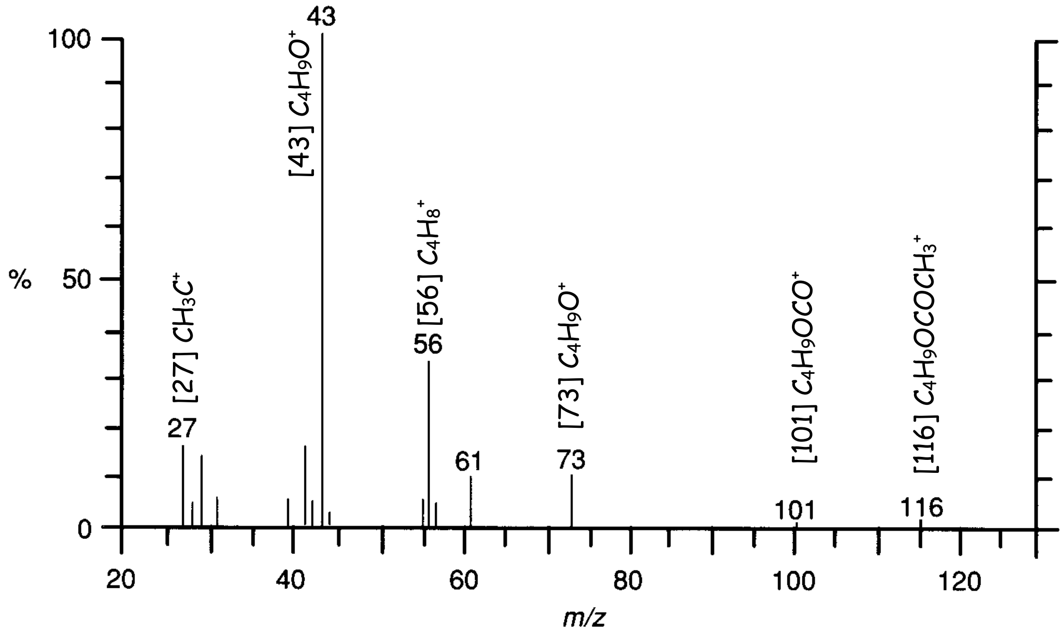
- Double-focussing mass spectrometers have an accuracy of up to 1×10^{-6}amu.

Formula	Molecular Mass (amu)	Exact Mass (amu)
$C_4H_4N_5$	122	122.046668
$C_4H_{10}O_4$	122	122.057903
$C_6H_4NO_2$	122	122.024201
$C_6H_6N_2O$	122	122.048010
$C_6H_8N_3$	122	122.071819
C_7H_8NO	122	122.060585

e.g. Mass Spectrum of Propanone CH_3COCH_3

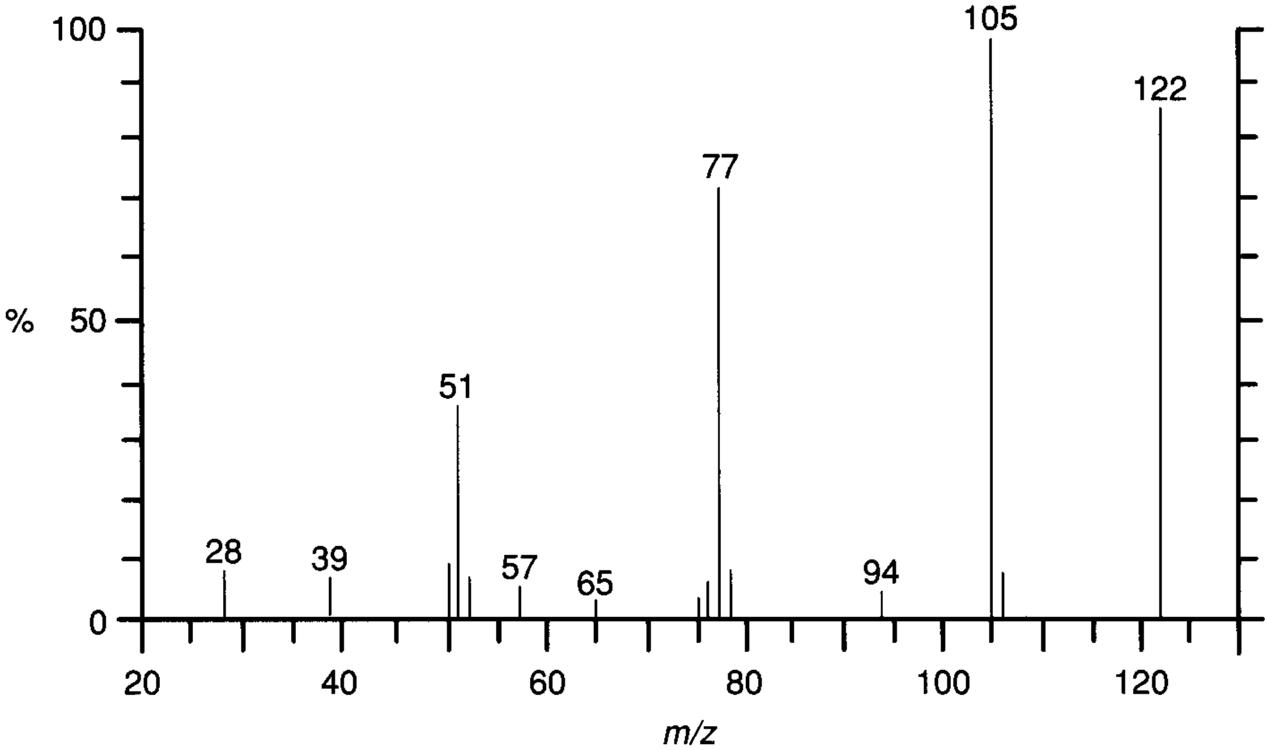


e.g. Mass Spectrum of butyl ethanoate $C_4H_9OCOCH_3$

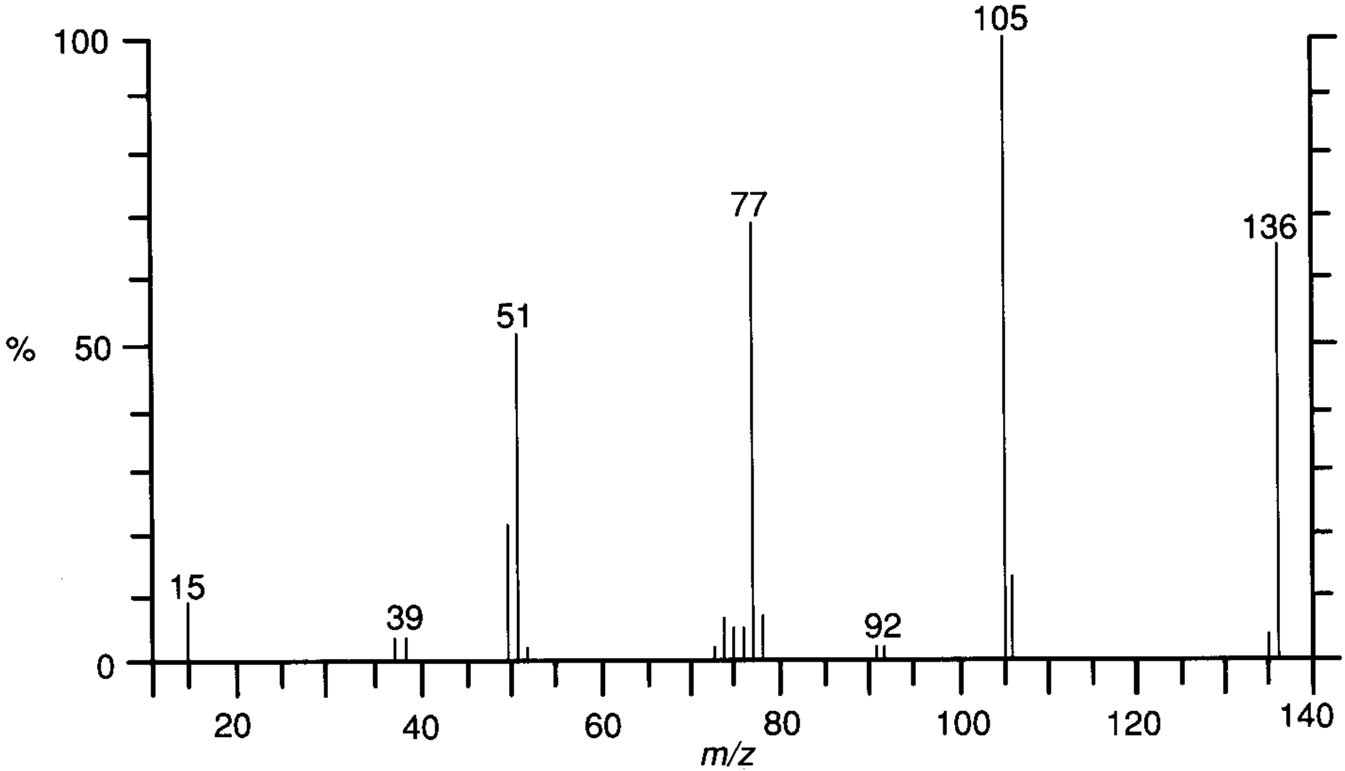


Questions

1. Look at the mass spectra of benzoic acid and identify the ions responsible for the major peaks.



2. Look at the mass spectra of methyl benzoate and identify the ions responsible for the major peaks.



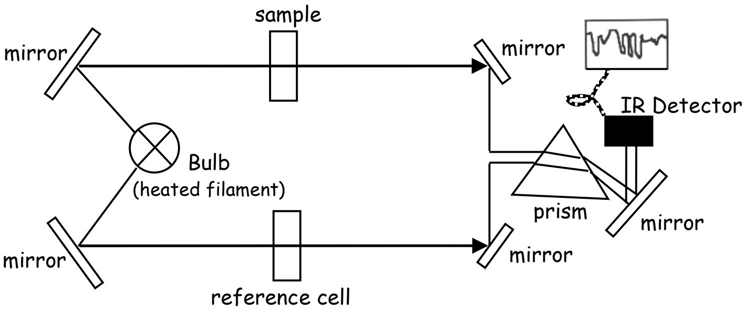
Infra-red Spectroscopy

Infra-red spectroscopy can be used to identify certain functional groups in organic compounds

- Different infra-red wavelengths cause certain parts of an organic molecule to vibrate
- Energy of wavelength is absorbed and causes vibration depends on
  - chemical bonds involved
  - atoms attached at either end of vibrating chemical group

The IR spectrometer works as follows:

1. IR radiation from a heated filament is split into two parallel beams
2. One beam passes through the reference cell, the other beam passes through the sample being investigated
3. Unwanted IR absorptions from water, CO_2 and solvent are removed by comparison of sample and reference sample
4. Beams are analysed by an IR detector for various frequencies after passing through a prism.
5. Where IR radiation has been absorbed by a chemical group, there is a decrease in the intensity between the sample and the reference cell. If there is no absorption, the sample and reference are identical.



The measurement of IR-absorbance by a chemical group is measured in wavenumber (units: cm^{-1})

$$c = \lambda \times f$$

speed ($m\ s^{-1}$) — wavelength (m) — frequency (Hz or s^{-1})

and wavenumber $\bar{\nu} = {}^{1}/_{\lambda}$ = waves per meter (m^{-1})

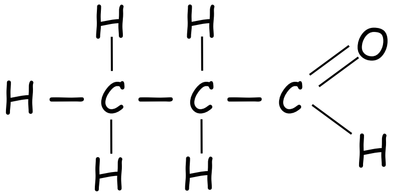
but most IR spectrometers are calibrated in cm^{-1}

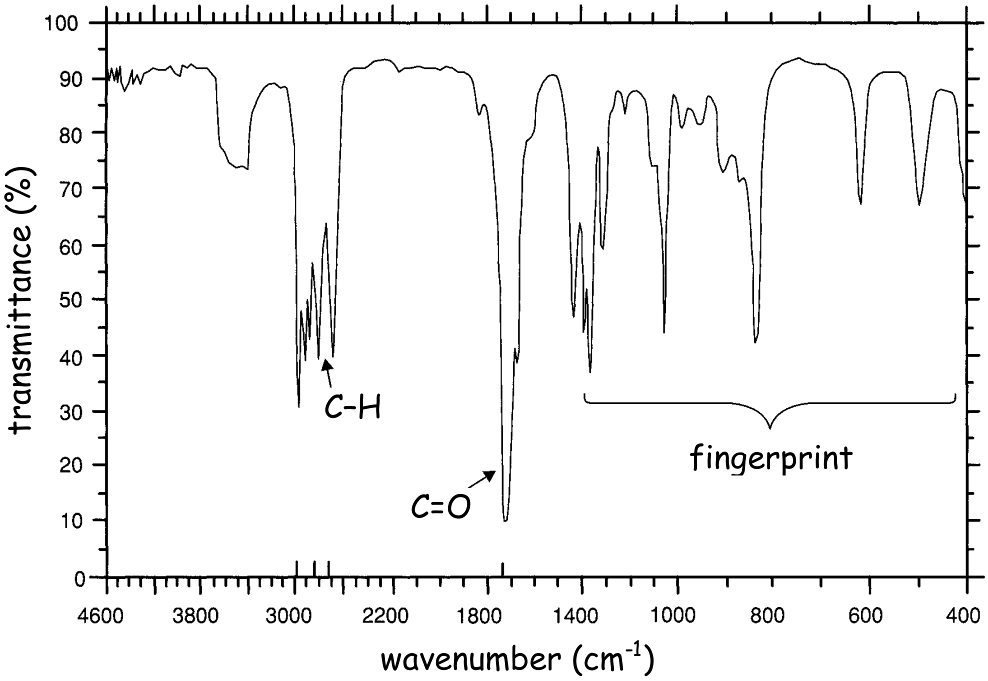
- multiply $\bar{\nu}$ in m^{-1} by 100 to change wavenumber into cm^{-1}
- $100m^{-1} = 1cm^{-1}$

NB: IR radiation has

- higher wavelength than visible light
- lower frequency than visible light
- same velocity as visible light
- lower wavenumber than visible light
- lower energy than visible light

e.g. IR Spectrum of Propanal





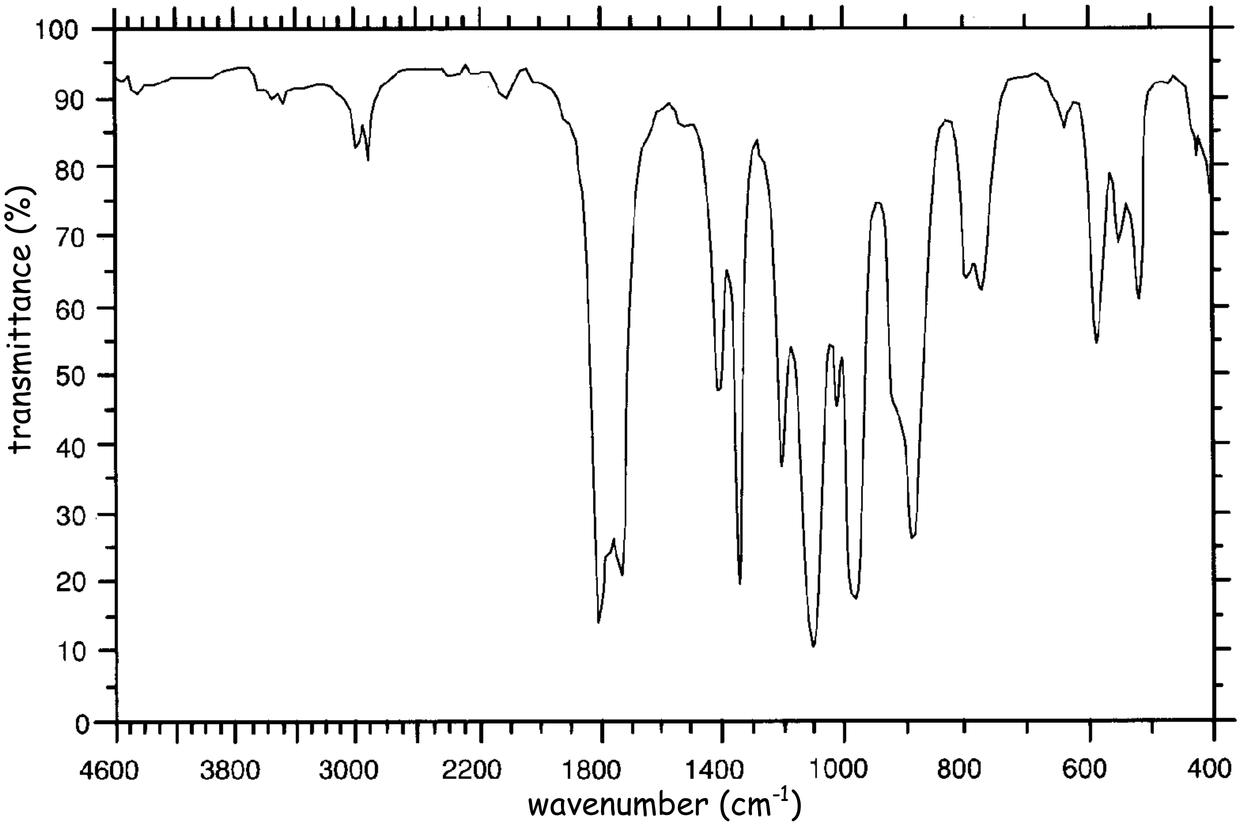
- major absorbance peak at 1730cm^{-1}
  - this is due to the C=O group stretching

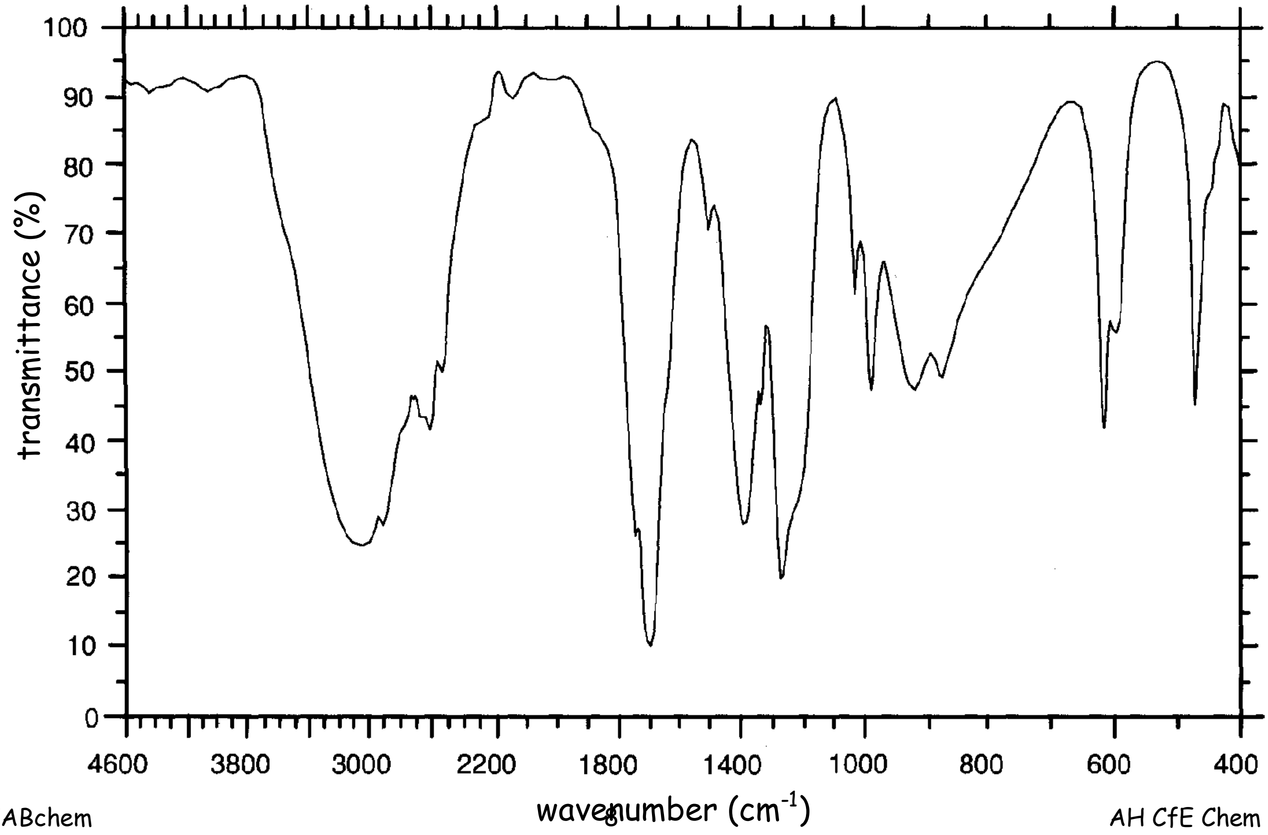
- major absorbance peak(s) just below 3000cm^{-1}
  - this is due to the C–H bonds stretching

- The various absorption peaks below 1400cm^{-1} are characteristic of a particular organic compound
  - Often called the *fingerprint* region of a particular compound
  - Computers store this data and can identify unknown organic compounds by using the *fingerprint* region.

Question

The spectra below are of ethanoic acid, CH_3COOH, and ethanoic anhydride, $(CH_3CO)_2O$. Draw the full structural formula for both compounds and then determine, giving reasons, which spectrum is due to which compound.





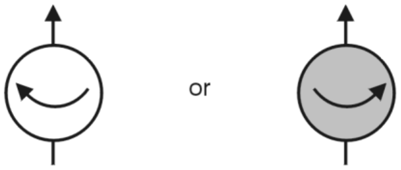
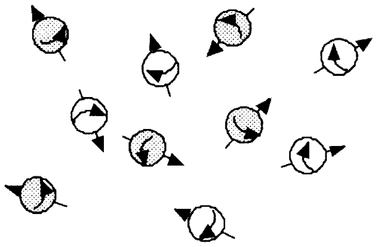
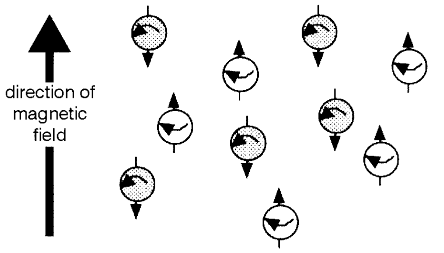
Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy can be useful in structural analysis as it gives information about

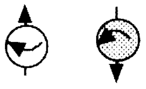
- The different environments of hydrogen atoms (i.e. chemical groups)
- The number of hydrogen atoms with in these environments

Protons, or in this case hydrogen nuclei, have a spin property. This spin property is similar in a way to the spin properties of electrons (Unit 1.2)

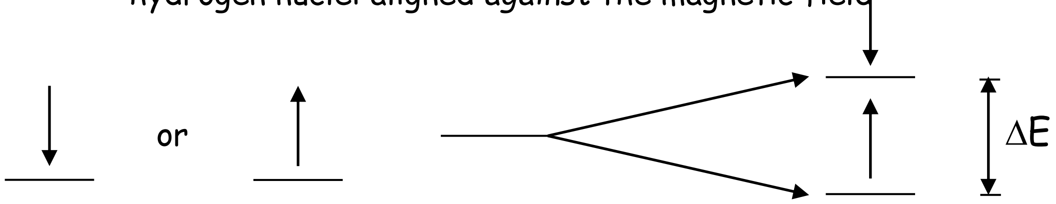
- Due to the spin property, hydrogen nuclei behave like tiny magnets themselves in a strong magnetic field
- Nuclei align themselves with the external magnetic field much like a compass needle does in the Earth's magnetic field

 Hydrogen nuclei have 2 possible spin orientations	 No Magnetic Field Hydrogen Nuclei not aligned	 Strong Magnetic Field Hydrogen Nuclei spins line up with magnetic field.

- There are two possible spin states for hydrogen nuclei
- In the absence of a strong magnetic field, hydrogen nuclei are not aligned in any particular direction
- In the presence of a strong magnetic field, hydrogen nuclei align in the same direction
  - There are still 2 spin directions for these aligned hydrogen nuclei
  - Some hydrogen nuclei are spinning *with* the magnetic field
  - Some hydrogen nuclei are spinning *against* the magnetic field



Those hydrogen nuclei aligned *with* the magnetic field are of lower energy than the hydrogen nuclei aligned *against* the magnetic field



Hydrogen nuclei can be aligned with or against the magnetic field

Spin against magnetic field has higher energy than spin with the magnetic field

The difference in energy ΔE between hydrogen nuclei spinning with and against the magnetic field lies in the radiofrequencies of the electromagnetic spectrum

- If radio waves are supplied, lower energy hydrogen nuclei can be *flipped* to the higher energy hydrogen nuclei
    - All nuclei are now spinning with magnetic field
- As these excited hydrogen nuclei fall back to their ground state radio waves are emitted and can be detected

Hydrogens in organic compounds have no electrons spinning around the nucleus as the electron is part of covalent bond

- Electrons spinning around nucleus would shield the spin effect of the nucleus
- In organic compounds, the electron density varies according to the order of bonds in the molecule
    - The hydrogen nuclei experience slightly differing magnetic fields at different parts of the molecule
    - The ΔE energy gaps vary from part of the molecule to part of the molecule depending on the local environment of the hydrogen nuclei

The variation in emitted radio waves (caused by the variation in ΔE) is called **chemical shift**.

- Dependant on the environment the hydrogen is in
- When the ^1H NMR spectrum is produced, the area under the peaks produced on the NMR are proportional to the number of hydrogen nuclei present

A standard reference chemical is added to the sample

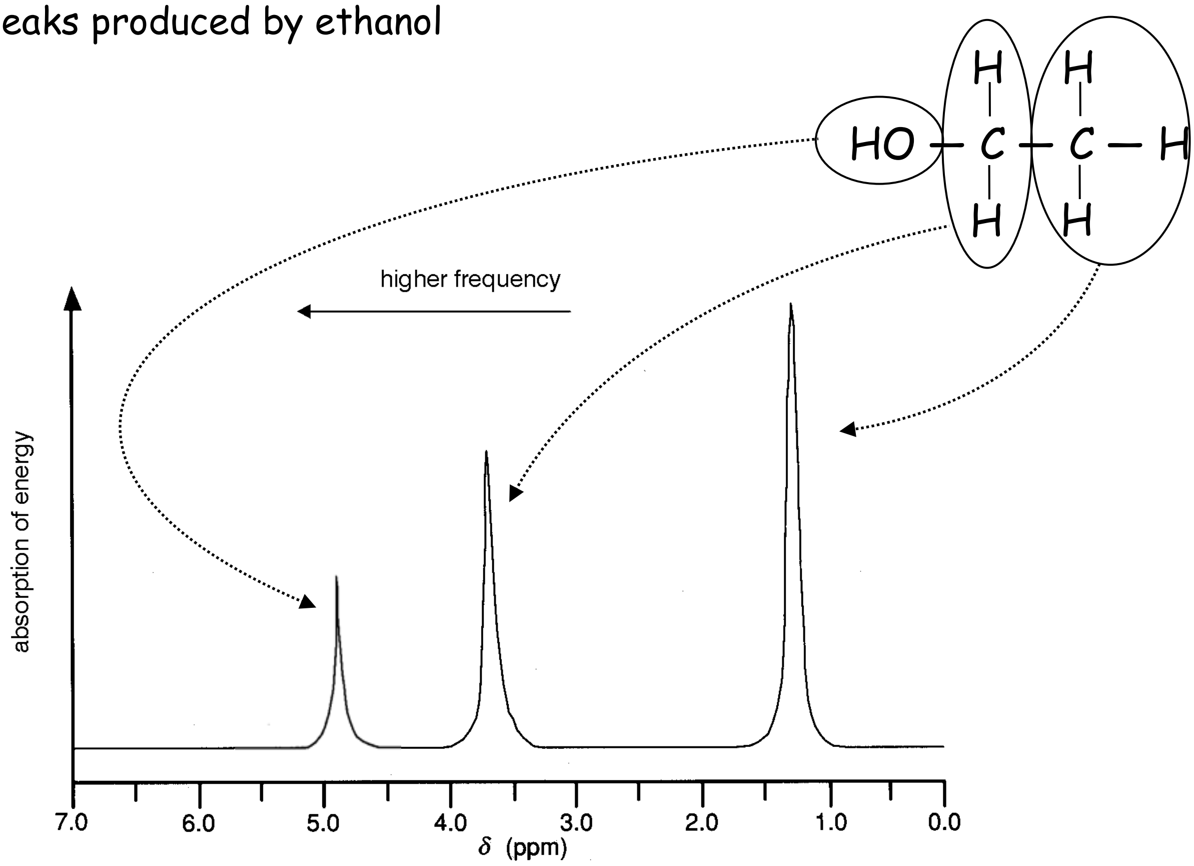
- Tetramethylsilane TMS [$Si(CH_3)_4$] is added
- TMS is assigned a chemical shift equal to zero
- Chemical shift is the difference between the sample's signal and the signal from TMS
- Chemical shift is donoted by the symbol delta (δ)
    - measured in ppm (parts per million)
    - chemical shift has a very small effect

Interpreting ^1H NMR Spectra

Hydrogen nuclei in different environments have different effects on the spectra produced

- in benzene C_6H_6, all hydrogens atoms are identical
    - only one peak appears on the NMR spectrum produced
- in ethanal CH_3CHO, there are two different environments for the hydrogen nuclei
    - one peak for the three hydrogens in the methyl $-CH_3$ group
    - another peak for the single hydrogen in the aldehydes $-CHO$ group

- 3 peaks produced by ethanol



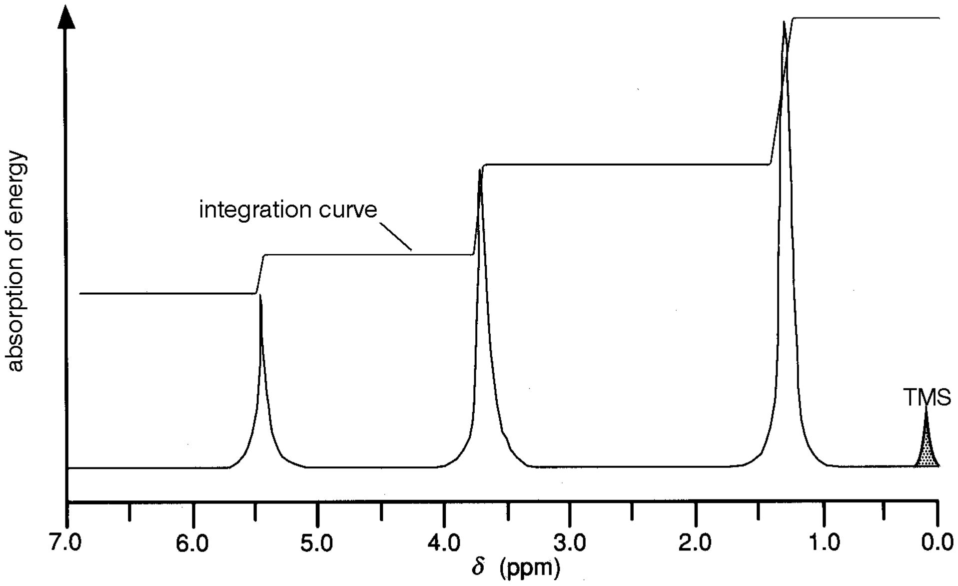
- peak at around δ=1.2 caused by H atoms in $-CH_3$ group
    - tallest peak due to 3 atoms of hydrogen in group
- peak at around δ=3.7 caused by H atoms in $-CH_2-$ group
    - medium peak due to 2 atoms of hydrogen in group
- peak at around δ=4.9 caused by H atom in $-OH$ group
    - smallest peak due to only 1 atom of hydrogen in group

The area under the peak is proportional to the number of hydrogen atoms absorbing radio waves

- area under the methyl peak is 3x the area under the hydroxyl peak

Estimation of area under peaks is difficult in practice

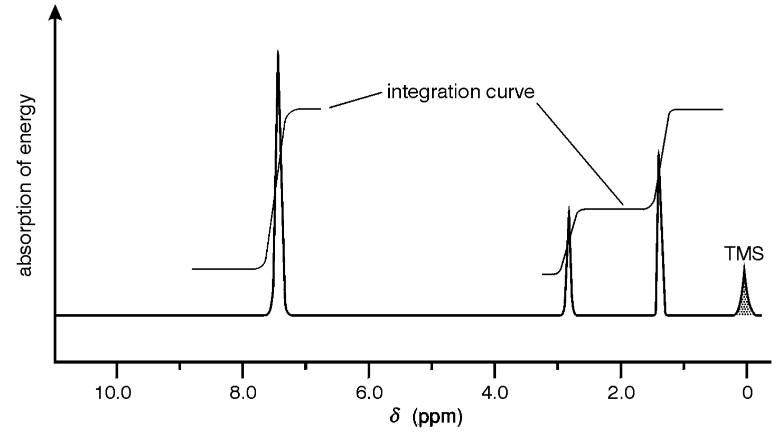
- integration curve is then plotted onto the NMR spectrum



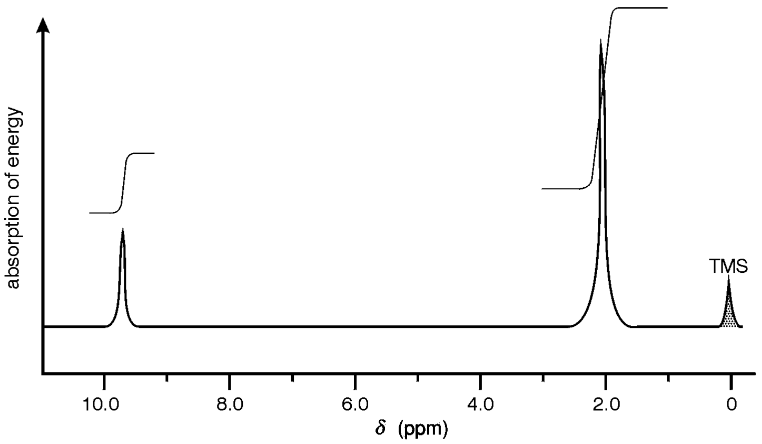
- by measuring the height of each *step* on the integration curve, the ratio of ^1H atoms can be determined

Questions

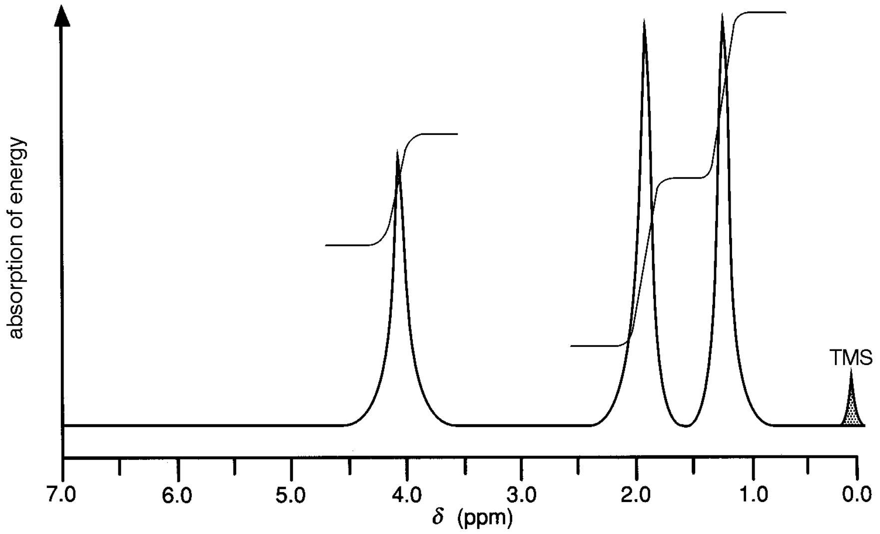
1. What is the structural formula for the hydrocarbon with the following NMR spectrum?



2. What is the structural formula for the C_2H_4O compound with the following NMR spectra?

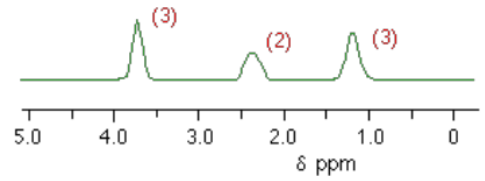
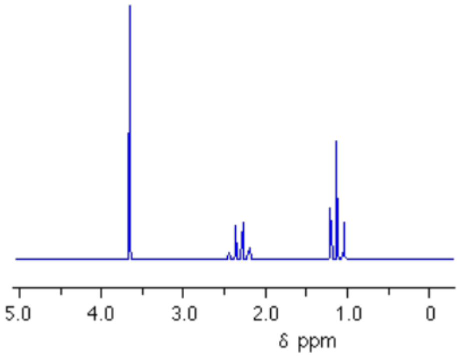


3. Analysis of a sweet-smelling, neutral compound of carbon, hydrogen and oxygen produced the following results: %C = 54.5% and %H = 9.1%.

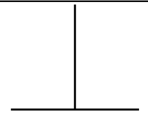
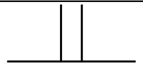
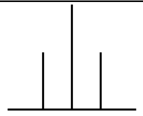
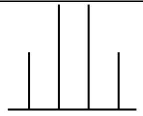
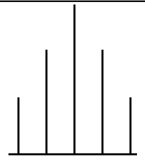


High Resolution NMR

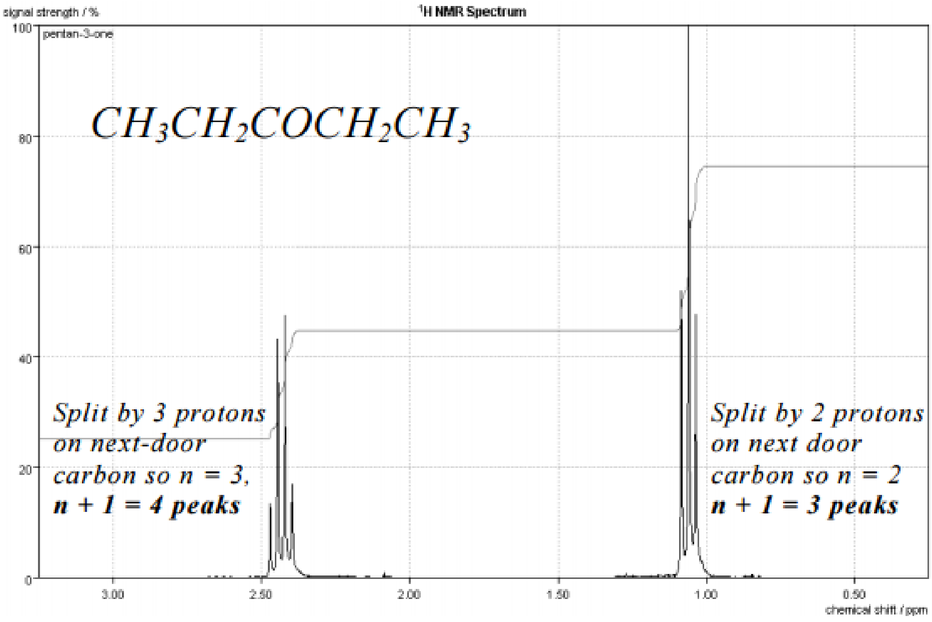
High-resolution spectra are run using a higher radio frequency and the peaks have more detail.

	
low resolution spectra methyl propanoate	high resolution spectra methyl propanoate

Under higher resolution, the peak arising from a particular hydrogen atom may be split into a double, triple or multiple peak. This provides a great deal of information about the structure of the molecule.

				
singlet no adjacent hydrogens	Doublet 1 adjacent hydrogen	Triplet 2 adjacent hydrogens	quadruplet 3 adjacent hydrogens	quintuplet 4 adjacent hydrogens

The hydrogens on the neighbouring carbon atoms cause the splitting of peaks. The splitting follow a simple 'n + 1' rule, where a peak is split into a 'doublet' if there is one adjacent hydrogen, a 'triplet' if two adjacent hydrogens and so on.



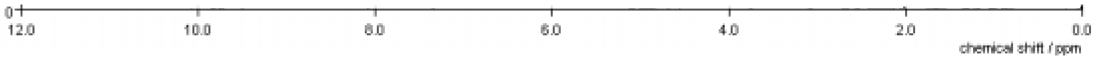
Not only can we tell how many different environments, and how many hydrogens are in each environment, we can also tell how many hydrogen are on the adjacent carbons. This is usually enough to help confirm the structure of a molecule.

<u>Question 1</u> For propan-1-ol

a) draw the full structural formula
b) circle the different proton environments.
c) copy and complete the following table using values in data booklet Group

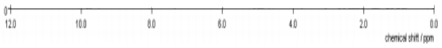
Group	Chemical Shift (ppm)

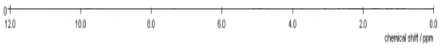
d) draw the low resolution NMR spectrum you would expect to see.
(Remember to draw in the appropriate peak heights – you can assume the peak height is the same as the area under the curve if the peak is very narrow.)

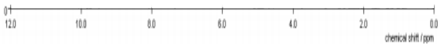


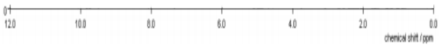
Question 2 Repeat the four steps in Question 1 for each of the following

a) propan-2-ol
b) butane
c) but-2-ene
d) methyl benzene





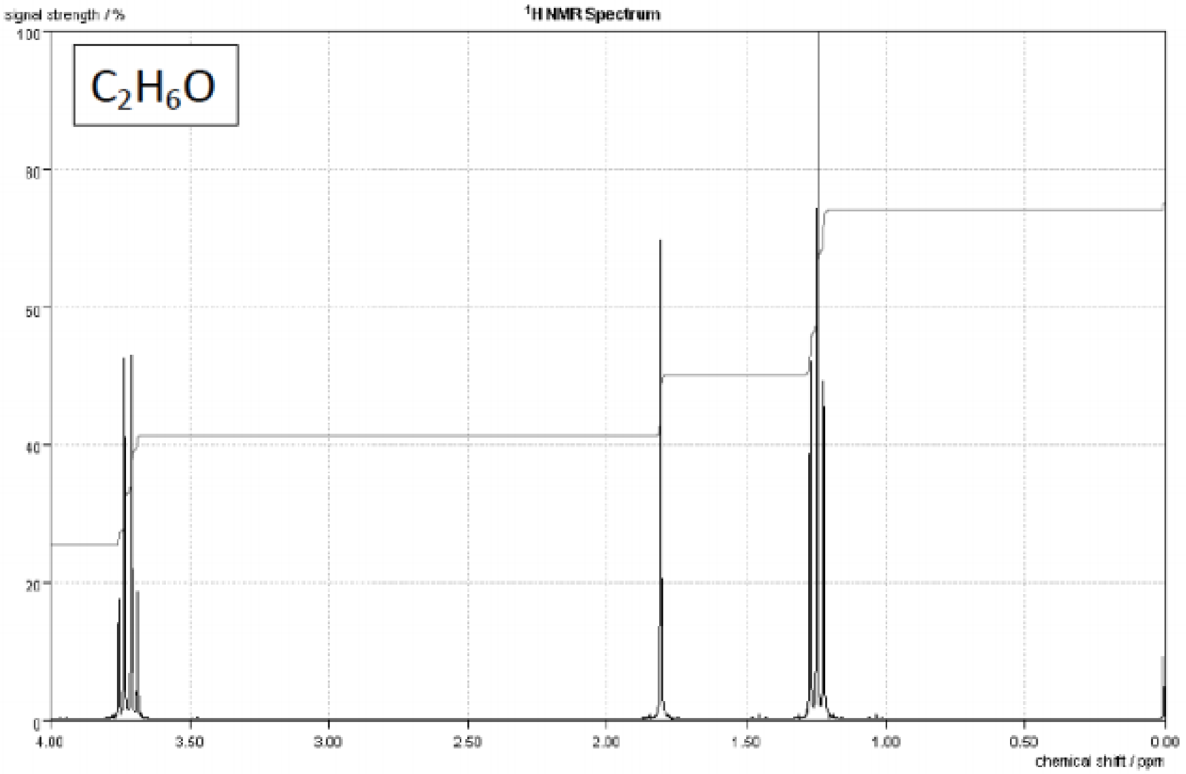




Question 3

For each of the following examples analyse the high resolution proton NMR spectrum and suggest a structural formula for each compound

Example 1

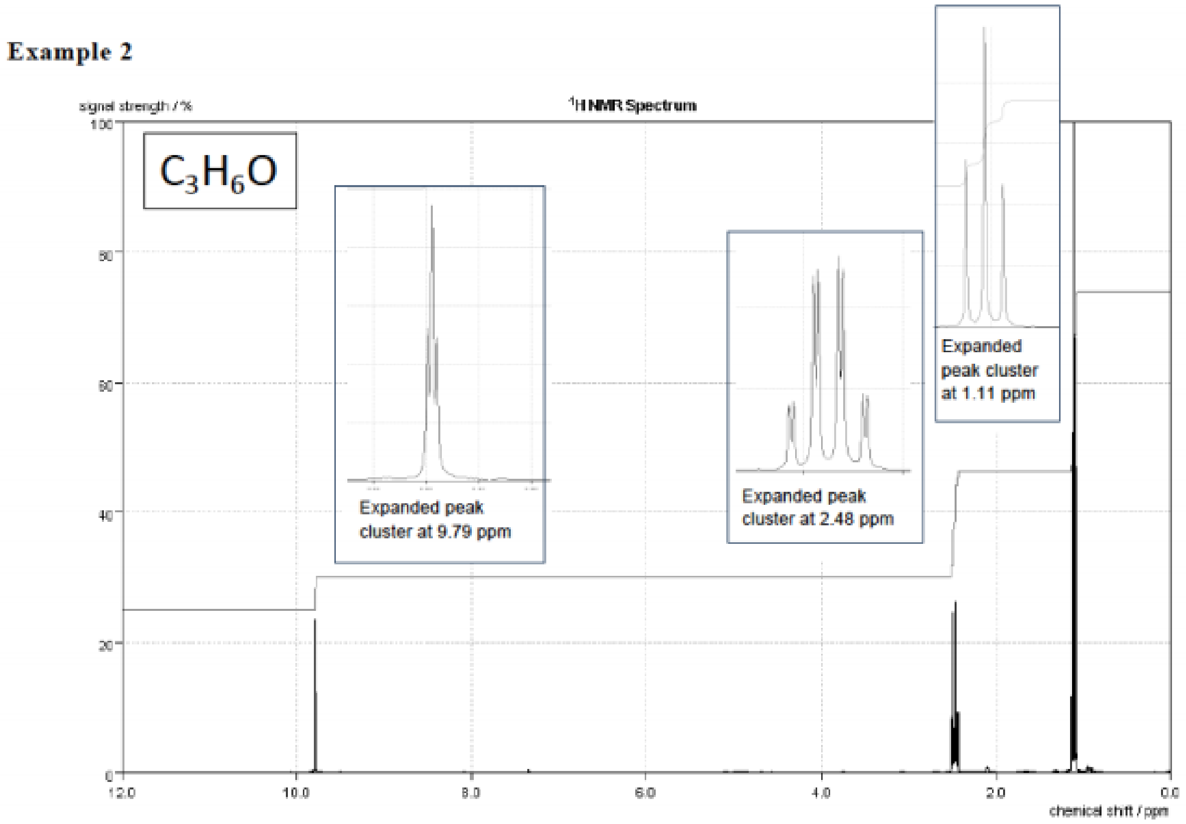


Peak	1st Peak δ=3.75	2nd Peak δ=1.8	3rd Peak δ=1.25
Possible Groups from chemical shift	☑ CH_3O-, RCH_2O-, R_2CHO- (alcohol, ether) ☒ $ArNH_2$, $Ar\overset{|}{N}H$ ☒ ROH ☒ CH_3X, RCH_2X, R_2CHX	☒ $CH_3\overset{|}{C}=\overset{|}{C}-$, $RCH_2\overset{|}{C}=\overset{|}{C}-$, $R_2CH\overset{|}{C}=\overset{|}{C}-$ ☒ RNH_2, $R\overset{|}{N}H$ ☑ ROH ☒ $CH_3C\equiv C-$, $RCH_2C\equiv C-$, $R_2CHC\equiv C-$ ☒ $-C\equiv CH$	☑ RCH_3, R_2CH_2, R_3CH ☒ RNH_2, $R\overset{|}{N}H$ ☒ ROH
Conclusion:	CH_3O-, RCH_2O-, R_2CHO- (alcohol, ether)	ROH	RCH_3, R_2CH_2, R_3CH
Height of Integration Curve	2	1	3
Conclusion:	RCH_2O-	ROH	RCH_3
No. of Peaks under high resolution	Quartet = 4	Singlet	Triplet
Conclusion:	3 H atoms on adjacent carbons	-OH groups does not give multiple peaks in Hi Res	2 H atoms on adjacent carbons
Overall	CH_3CH_2- O	R - OH	CH_3 – CH_2 -

Conclusion	$\underbrace{\qquad\qquad\qquad\qquad\qquad\qquad}_{CH_3CH_2OH}$

Example 2

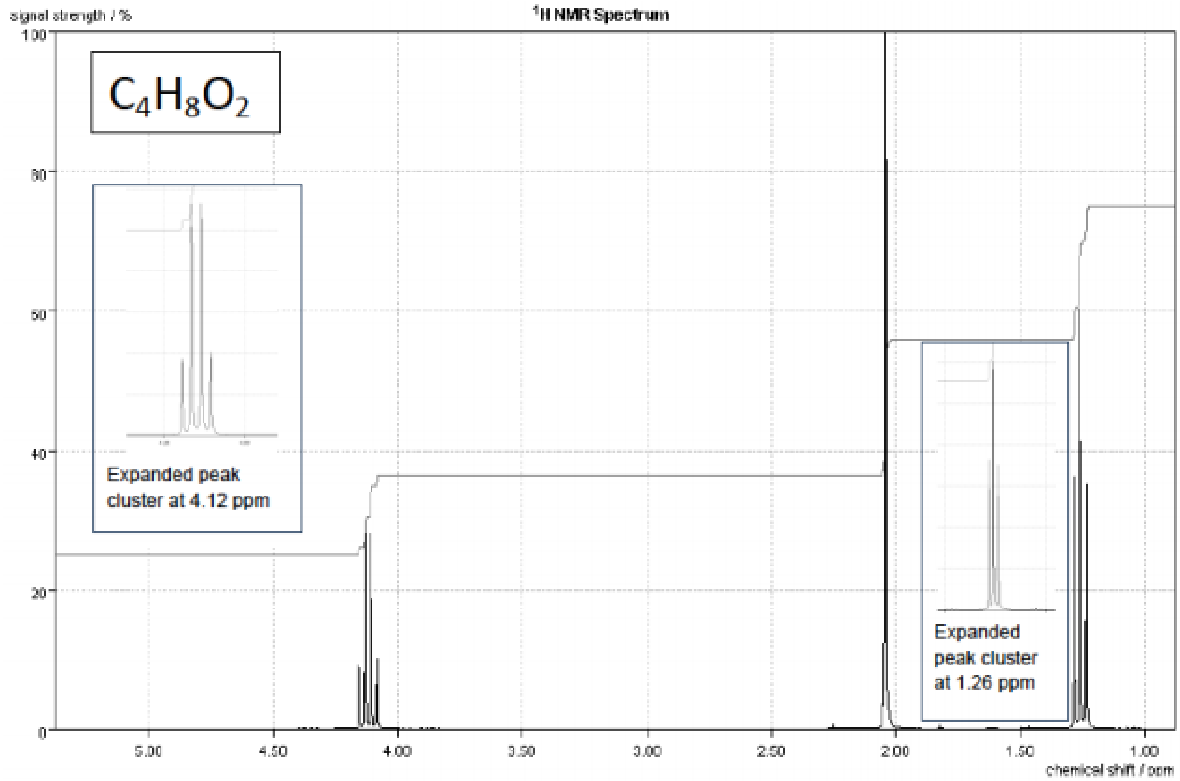
C_3H_6O could be propanal, propanone, prop-2-en-1-ol or prop-1-en-2-ol



Peak	1st Peak δ=9.8		2nd Peak δ=2.5		3rd Peak δ=1.2	
Possible Groups from chemical shift	☑	$R\overset{O}{\overset{\|}{C}}-H$	☒	$ArCH_3$, $ArCH_2R$, $ArCHR_2$	☑	RCH_3, R_2CH_2, R_3CH
	☒	$Ar\overset{O}{\overset{\|}{C}}-H$	☑	$CH_3\overset{O}{\overset{\|}{C}}-$, $RCH_2\overset{O}{\overset{\|}{C}}-$, $R_2CH\overset{O}{\overset{\|}{C}}-$ Aldehyde/ketone/acid/ester/amide	☒	RNH_2, $R\overset{\|}{N}H$
	☒	ArOH	☒	CH_3X, RCH_2X, R_2CHX	☑	ROH (if C=C double bond present)
			☒	CH_3CN, RCH_2CN, R_2CHCN		
			☒	$CH_3N\lt$, $RCH_2N\lt$, $R_2CHN\lt$		
			☒	$CH_3\overset{\|}{C}=\overset{\|}{C}-$, $RCH_2\overset{\|}{C}=\overset{\|}{C}-$, $R_2CH\overset{\|}{C}=\overset{\|}{C}-$		
Conclusion:	$R\overset{O}{\overset{\|}{C}}-H$		$CH_3\overset{O}{\overset{\|}{C}}-$, $RCH_2\overset{O}{\overset{\|}{C}}-$, $R_2CH\overset{O}{\overset{\|}{C}}-$ **Aldehyde**/ketone/acid/ester/amide		RCH_3, R_2CH_2, R_3CH	
Height of Integration Curve	1		2		3	
Conclusion:	$R\overset{O}{\overset{\|}{C}}-H$		$RCH_2\overset{O}{\overset{\|}{C}}-$		RCH_3	
No. of Peaks under high resolution	Singlet		Quartet		Triplet	
Conclusion:	No H atoms on adjacent carbons		3xH atoms on adjacent carbons		2xH atoms on adjacent	

			carbons
Overall Conclusion	$-CH_2-CHO$	CH_3-CH_2-CO-	CH_3-CH_2-
	CH_3CH_2CHO (propanal)		

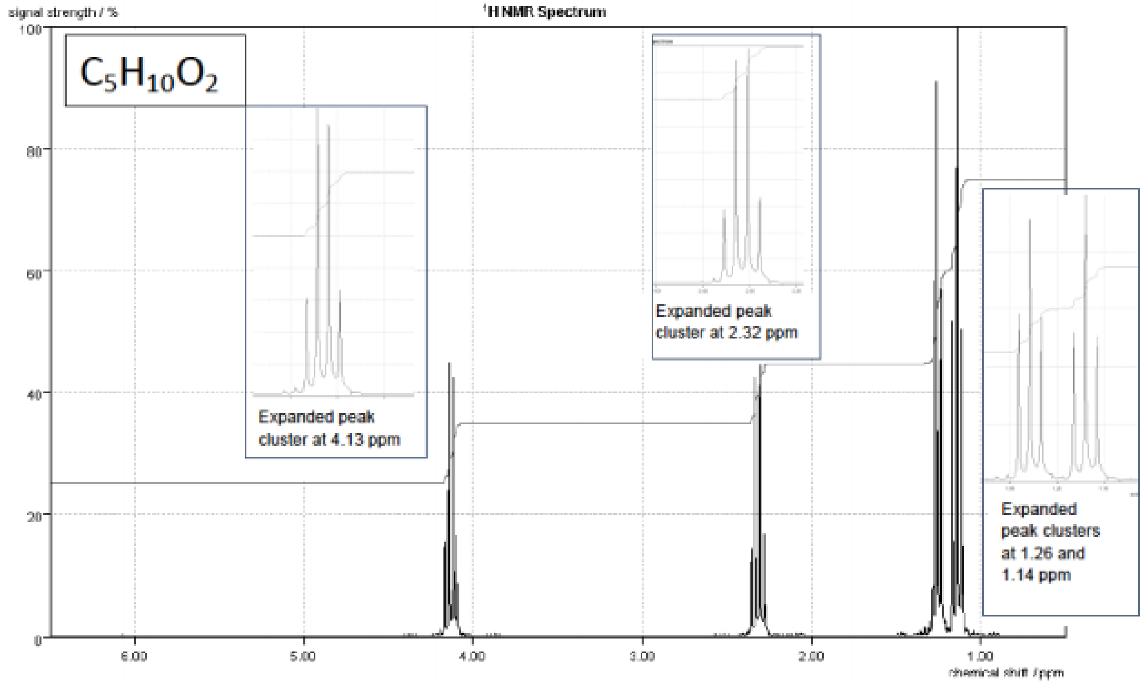
Example 3



Peak	1st Peak δ=		2nd Peak δ=		3rd Peak δ=	
Possible Groups from chemical shift						
Conclusion:						
Height of Integration Curve						
Conclusion:						
No. of Peaks under high resolution						
Conclusion:	xH atoms on adjacent carbons		xH atoms on adjacent carbons		xH atoms on adjacent carbons	
Overall						

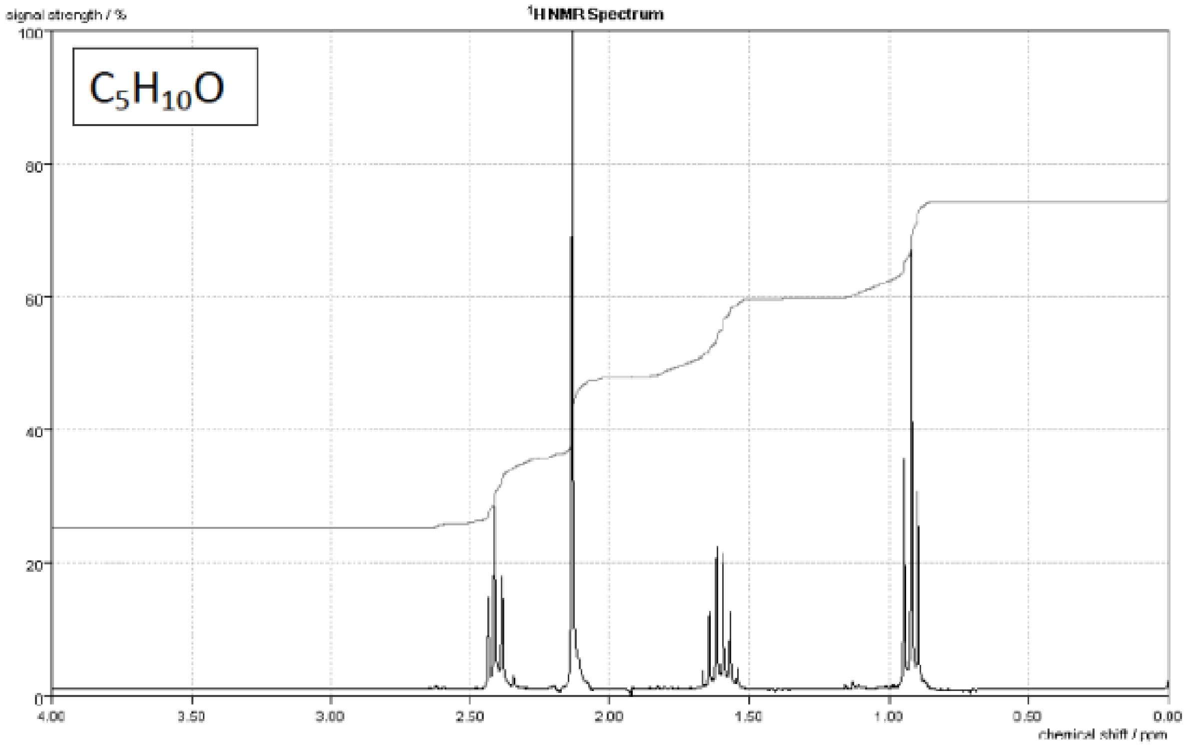
Conclusion	

Example 4



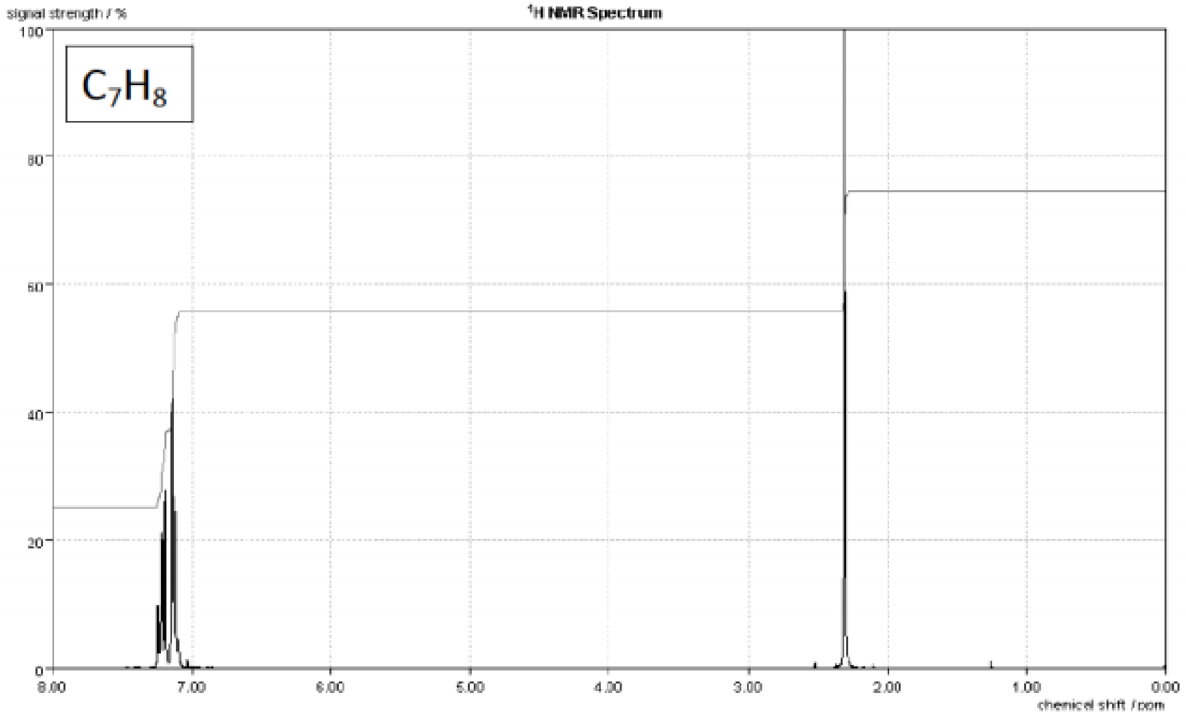
Peak	1st Peak δ=		2nd Peak δ=		3rd Peak δ=	
Possible Groups from chemical shift						
Conclusion:						
Height of Integration Curve						
Conclusion:						
No. of Peaks under high resolution						
Conclusion:	xH atoms on adjacent carbons		xH atoms on adjacent carbons		xH atoms on adjacent carbons	
Overall Conclusion						

Example 5



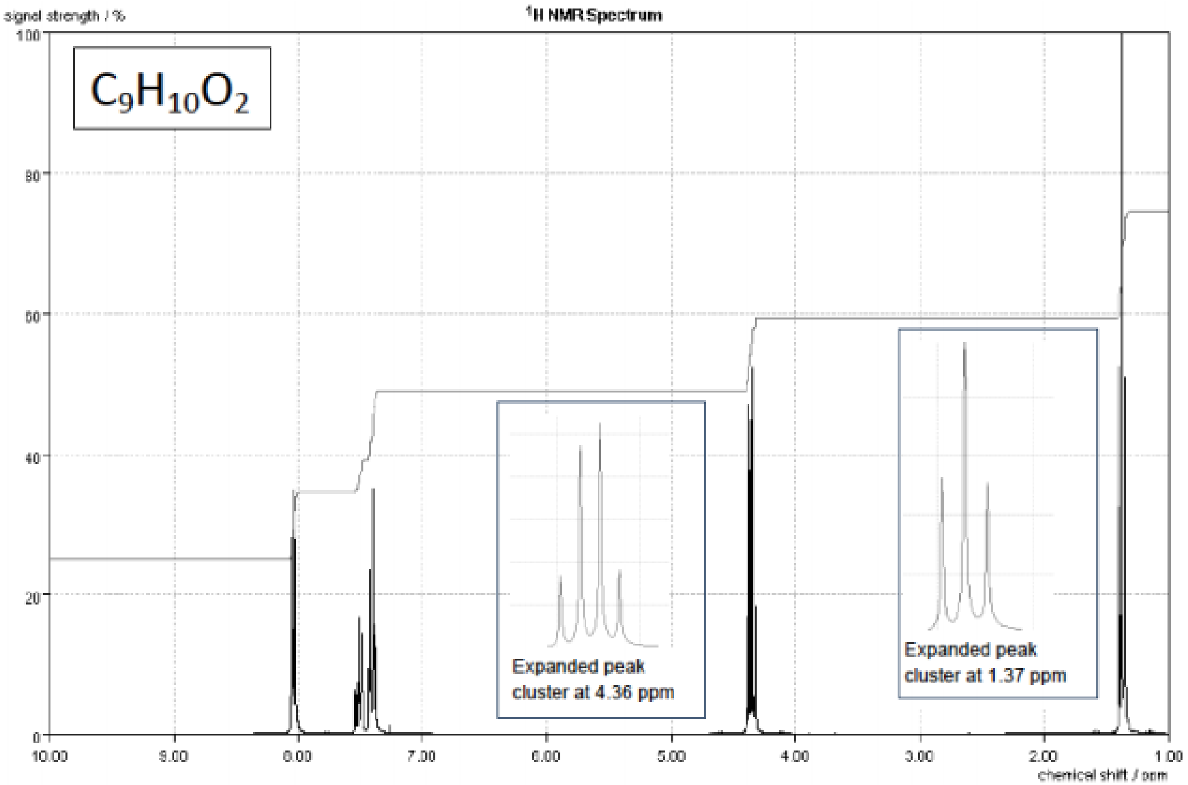
Peak	1st Peak δ=		2nd Peak δ=		3rd Peak δ=	
Possible Groups from chemical shift						
Conclusion:						
Height of Integration Curve						
Conclusion:						
No. of Peaks under high resolution						
Conclusion:	xH atoms on adjacent carbons		xH atoms on adjacent carbons		xH atoms on adjacent carbons	
Overall Conclusion						

Example 6



Peak	1st Peak δ=		2nd Peak δ=		3rd Peak δ=	
Possible Groups from chemical shift						
Conclusion:						
Height of Integration Curve						
Conclusion:						
No. of Peaks under high resolution						
Conclusion:	xH atoms on adjacent carbons		xH atoms on adjacent carbons		xH atoms on adjacent carbons	
Overall Conclusion						

Example 7



Peak	1st Peak δ=	2nd Peak δ=	3rd Peak δ=
Possible Groups from chemical shift			
Conclusion:			
Height of Integration Curve			
Conclusion:			
No. of Peaks under high resolution			
Conclusion:	xH atoms on adjacent carbons	xH atoms on adjacent carbons	xH atoms on adjacent carbons
Overall Conclusion			

X-Ray Crystallography

X-ray crystallography is one of the most powerful tools for determining the precise 3-D structure of organic compounds

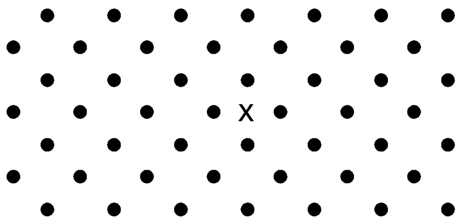
- Computers have transformed the technique in recent years

X-ray crystallography is based on diffraction

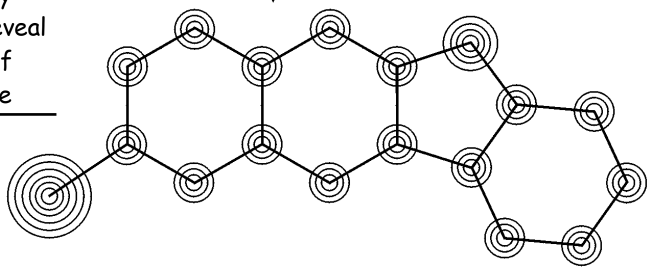
- Diffraction is when waves bend as they pass objects
- The amount of bending depends on
  - The wavelength of the waves
  - The size of the gap between objects
- When the size of the gaps and the wavelength of the waves are approximately equal, diffraction is at its greatest
  - Spacing of atoms in organic molecules is similar to the wavelength of X-rays
  - Wavelength of X-rays: ~100pm
  - 100pm = 0.1nm = 1×10^{-10}m = 1Å (Angstrom)

A beam of X-rays of a single wavelength are passed through a substance

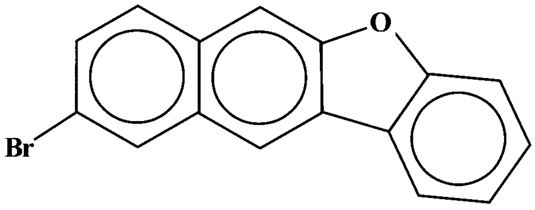
- Substance acts as a 3-D diffraction grating
- X-ray beam is transmitted as a number of diffracted beams
- A combination of diffraction and interference gives a spot spectrum
- Computers analyses the data produced and electron density maps for the molecule are produced



Spot spectrum is interpreted by computer to produce electron density diagram



Electron density diagram used to reveal the structure of organic molecule

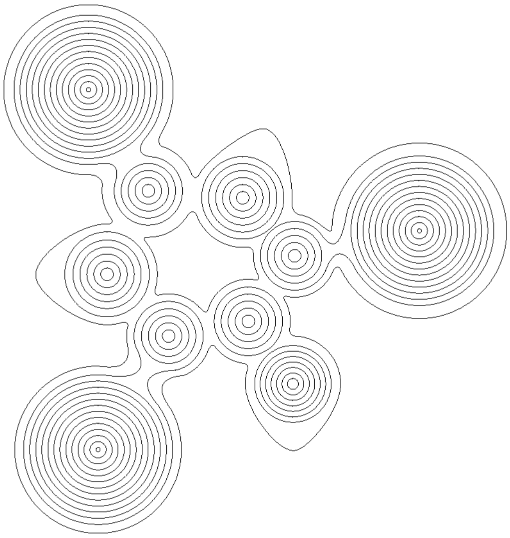


From electron density maps

- precise location of each atom can be determined
- heavier atoms have more electrons and show as electron-dense regions on electron density maps
- hydrogen atoms have low electron density
    - not easily detected by X-ray Crystallography
    - hydrogen atoms barely show on electron density maps

Questions

1. The following diagram is the simulated electron density map of an aromatic compound with molecular formula $C_6H_3Cl_3O$.



    a) Draw the full structural formula for this compound.
    b) Explain why you drew the structure in terms of electron density.

2. Draw an electron density map for methanoic acid.