

Lecture 7 - How do you weigh a molecule?

Background content

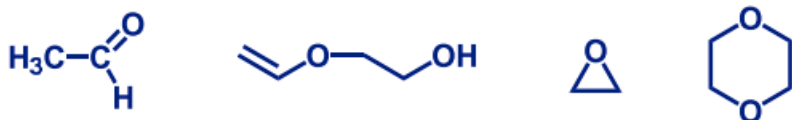
There are a number of stages involved with determining the molecular structure of an unknown compound:

1. Isolate and purify it
 - Precipitation, distillation, recrystallisation, chromatography etc
2. Determine molecular formula
 - Mass spectrometry
3. Identify functional groups and conjugation
 - Infrared and UV/Vis spectroscopies
4. Assemble structural formula
 - With correct bond connectivity and stereochemistry (NMR spectroscopy)

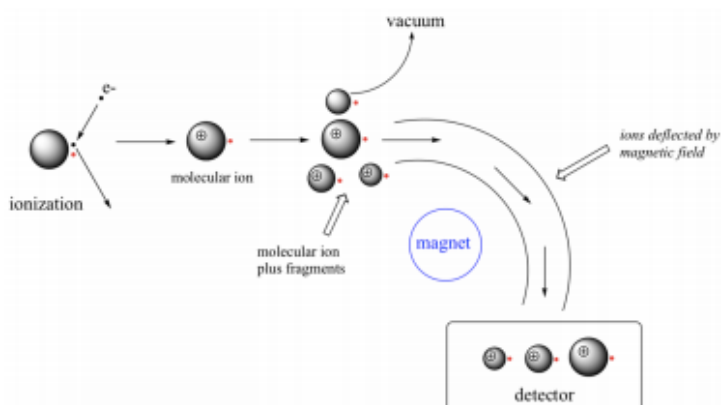
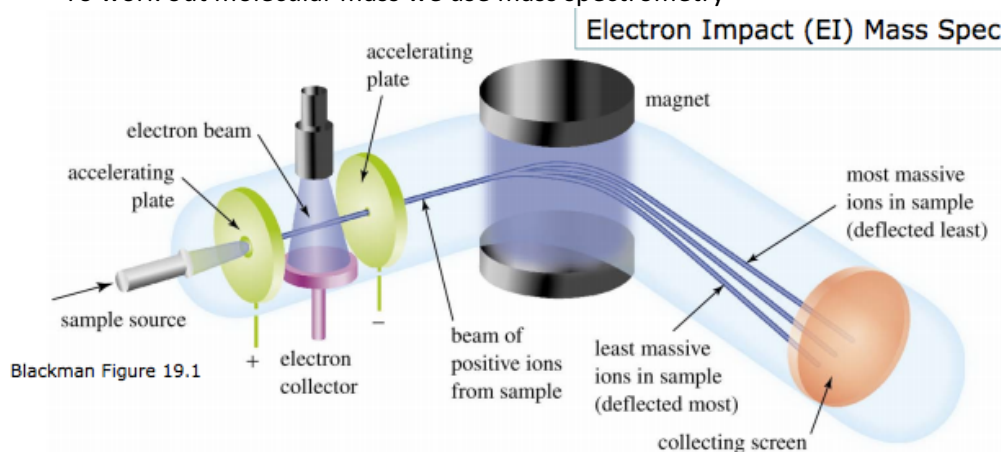
***Down the list is increasingly useful for determining structure

Empirical Formula - Combustion analysis

- React organic compound with O₂ and measure how much CO₂ and H₂O is released.
 - Carbon and hydrogen are always present
 - If N, S, P, halogens present then these will be shown as byproducts
 - BUT cant easily measure O (as reacting with O₂) so it is assumed to make up the difference to 100%.
- **Problems:**
 - An empirical formula just shows the relative ratios of atoms and their molecular masses and molecular formulae are different. Eg C₂H₄O can give:



To work out molecular mass we use mass spectrometry



- Molecules are ionised by impact with electrons in a vacuum ie. An electron is taken off and they are turned into charged species (ion). This is typically just +1.
- Some ions fragment to form smaller ions (**Daughter ions**)
- These ions are then accelerated through an electric field.
- Depending on the size of the molecule and its charge it will bend in different ways through the magnet. These tiny deflections are read by a collecting screen.

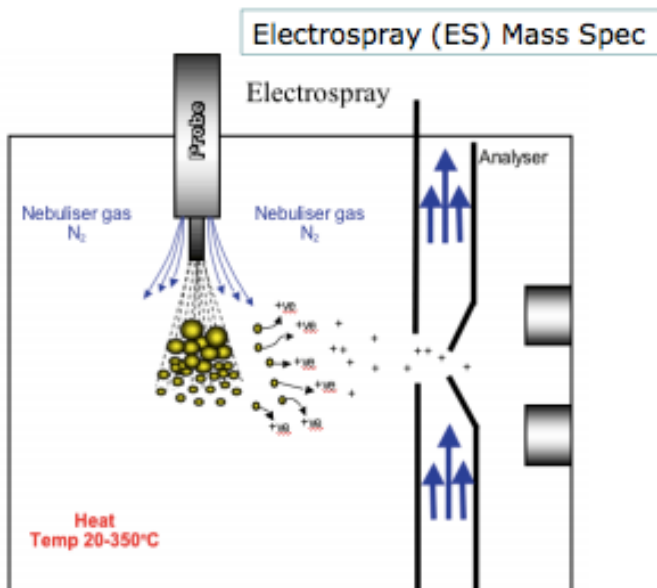
Another explanation:

- Ions are separated according to m/z (mass/charge)
 - Beam of ions directed into magnetic field, causing ions to bend
 - They trace out circular paths, with radii dependent on m and z ... but charge (Z) is almost always 1.
 - Detector measures how far ions have bent, so can work out m .

Mild method of ionisation- Electrospray Ionisation

ELECTROSPRAY IONISATION: more mild and gentle form of mass spectroscopy (rather than electron bombardment) that uses a mild acid/buffer to form protonated ions (MH^+ as oppose to M^+).

- Ions enter mass analyser as before
- Very gentle -large molecules don't break down as much (less daughter ions) so can be used to analyse proteins.



Identify molecular ion, base peak and daughter ions

Mass Spectrometry

Molecular ion:

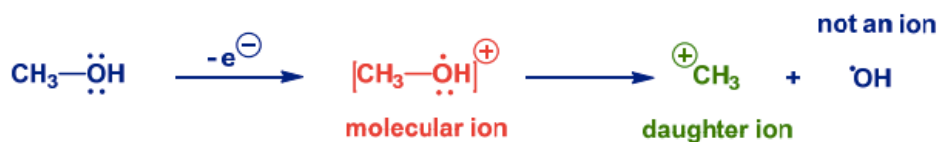
- The heaviest ion possible
- Results from loss of one electron from the parent molecule (electron mass is so light that the mass is negligible hence molecular ion has same as parent molecule).

Daughter ions:

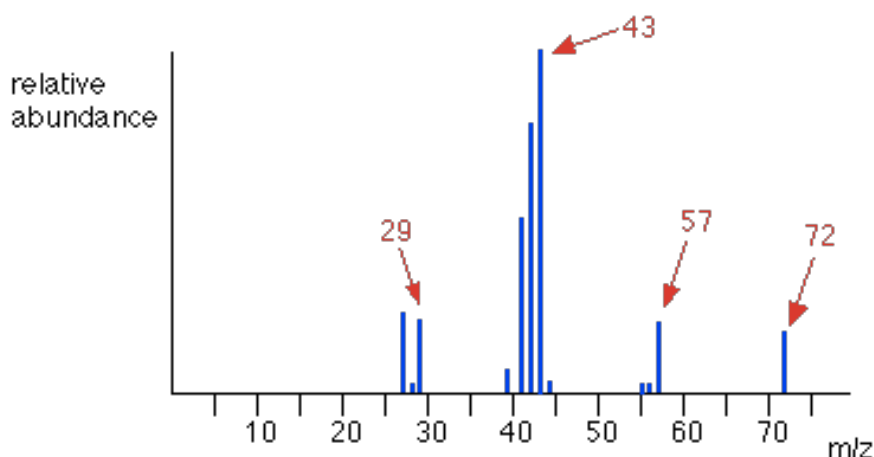
- Fragmentations of the parent molecule
- Help in determining the structure of the molecule but molecular ions MORE USEFUL

Base Peak:

- A typical mass spectrum has a molecular ion and many daughter ions. The base peak is the daughter ion that is more abundant i.e most intense signal, intensity is set to 100%



simplified mass spectrum of pentane - $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$



***The most abundant ion found is C_3H_7 which is the base peak shown with molecular weight 43 (m/z). (worked out by guessing formulae of C_xH_y until molecular weight = 43)

- The furthest right peak (72) is the molecular ion - ie heaviest molecular ion and would be the molecular mass of pentane.

Determine the m/z of a compound from its mass spectrum

- Mass spectrometry measures the mass:charge ratio (m/z ratio) of individual ions, including the molecular ion (i.e the ionised form of the molecule)
- This effectively allows us to "weigh" individual molecules, giving molecular weight information which we can deduce a molecular formula.

Identify some fragmentation/isotope information from mass spec

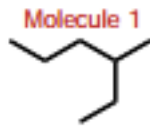
- Whichever ionisation method is used, each ion recorded in the mass spectrum registers specific isotopes of the elements present in that individual ion.
- Many elements have only one isotope of high abundance but some elements have more than one abundant isotope



So any sample of bromobenzene contains near equal amounts of $\text{C}_6\text{H}_5^{79}\text{Br}$ and $\text{C}_6\text{H}_5^{81}\text{Br} \Rightarrow$ see two molecular ion peaks

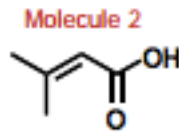
***if two almost equal molecular ion peaks (heaviest ion) then Bromine exists in the molecule.

- Isotopes have different number of neutrons, hence have different mass explaining why there are some high intensity readings for mass:charge ratios that are quite close in value e.g ^{13}C vs ^{12}C
- High resolution mass spectroscopies** helps to exactly determine the molecular mass of the compound (to 4 dec pl) -> where all substances have a unique mass.
 - High resolution mass spectrometry uses the most abundant isotopes to 4 decimal places to get more accurate answers -> needed if by chance 2 molecules have the same mass spectra m/z but have a different chemical formula e.g.



Chemical Formula: C_7H_{16}

Mass spectra m/z : 100



Chemical Formula: $C_5H_8O_2$

Mass spectra m/z : 100

Identify conjugation in a UV-vis spectrum

- UV/Vis Spectroscopy visualises electronic transitions within a molecule, so reveals the presence (or absence) of conjugation in an organic molecules.
- Conjugation lowers the energy gap between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), which is the gap across which an electron is excited by UV/Visible light to move from one orbital to another.

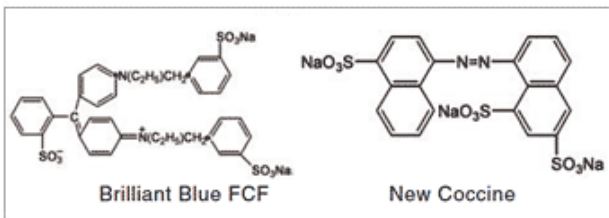
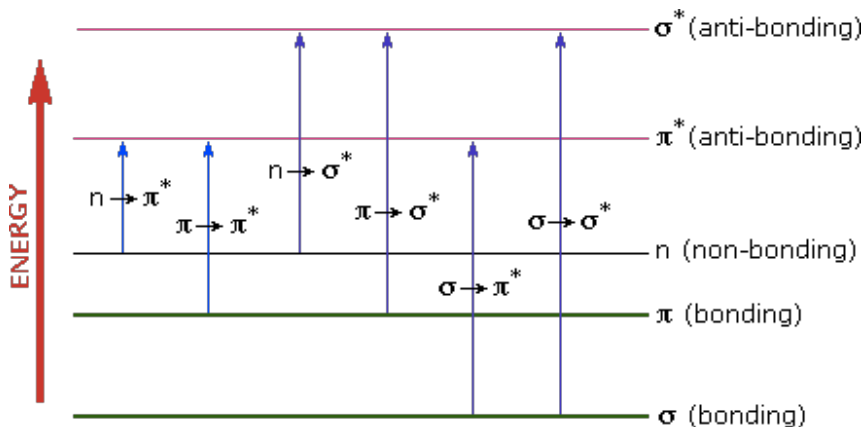


Fig.3 Structures of New Coccine and Brilliant Blue FCF

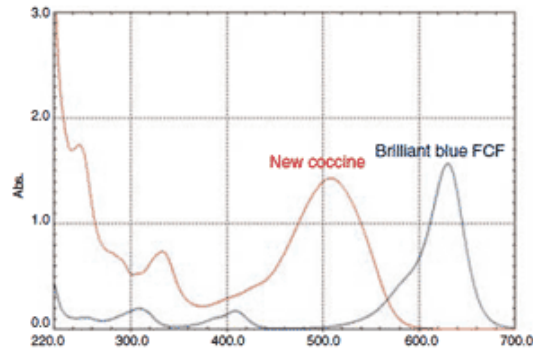


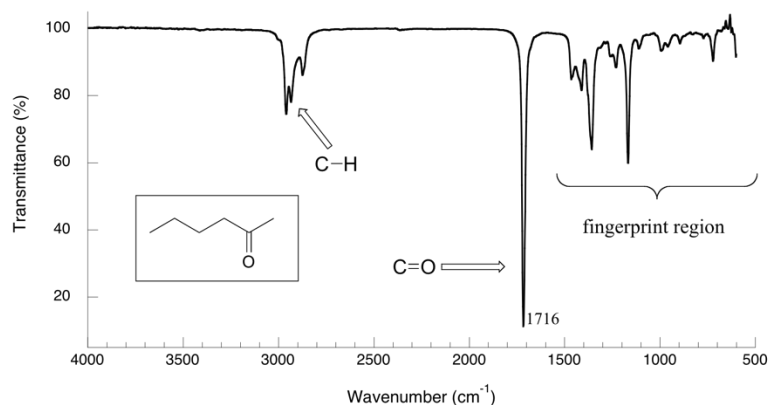
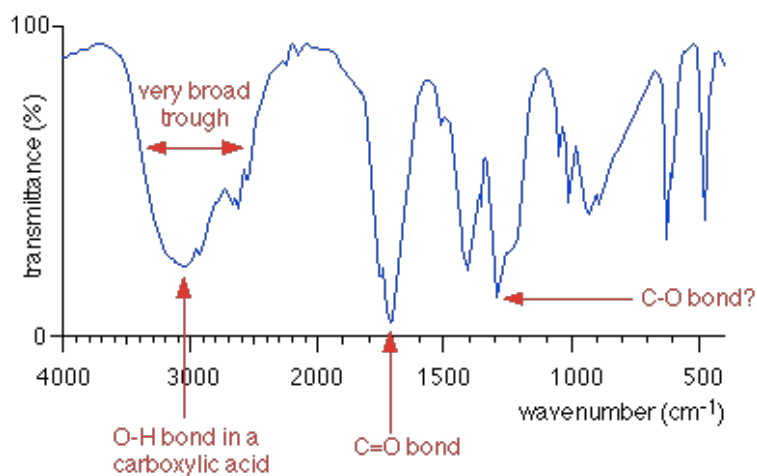
Fig.4 Absorption Spectra of Food Dyes
New Coccine and Brilliant Blue FCF

Identify functional groups from IR spectra

- IR spectroscopy visualises bond vibrations within a molecule (bonds excited after absorbing IR)
- Energy absorbed provides key signals in the IR spectrum for the presence (or absence) of particular functional groups

Bonds	IR absorption (cm^{-1})	
N-H	~3300	Amines, amides, alcohols, phenols, carboxylic acids
O-H (broad)		
C-H	~3000	
C=C	~2200	Ketones, aldehydes, esters, amides, carboxylic acids
C-N		
C=O	~1700	
C=C	~1600	

infra-red spectrum of ethanoic acid, CH_3COOH



Lecture 8 - ^{13}C NMR

Introduction:

Spectroscopy

- Involves absorption of part of the electromagnetic spectrum
- Triggers a change of energy level occupancy within the molecule
- The light absorbed is related to the process involved
- **Energy absorbed -> Signal in Spectrum -> STRUCTURAL INFO**

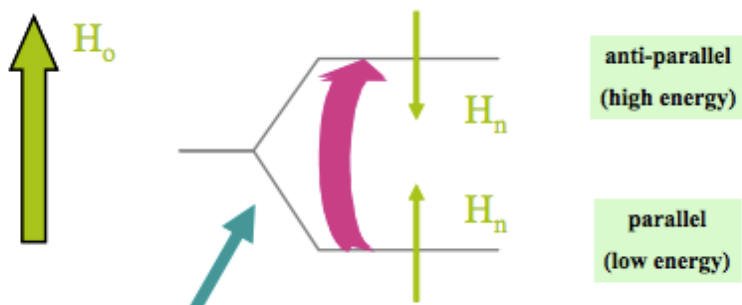
Understand the basic principles of NMR

Nuclear Magnetic Resonance (NMR) spectroscopy provides information on **structural connectivity**.

- Used in analysis and structure determination (NMR)
- Used to image soft tissue in animals (MRI) which looks at H₂O concentration in different parts of the body for abnormalities.

Principles of MR Spectroscopy

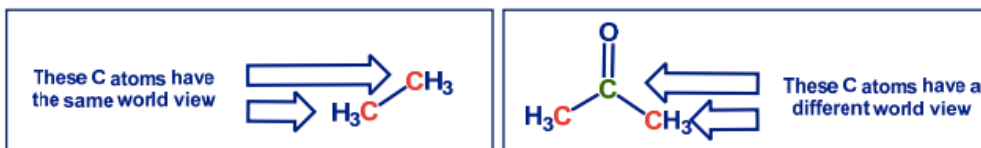
- Uses energy in the radio frequency region
- Some atomic nuclei behave like "mini-magnets" (because nuclei are charged) -> When put into external magnetic field, they align parallel or anti-parallel to it. ¹³C, ³¹P, ¹⁹F and ¹H behave this way, hence are NMR active.
- There is an energy difference between the two alignments
- Radio waves make low energy spin "flip" to high energy status



Energy absorbed \Rightarrow signal in spectrum \Rightarrow structural info

Identify the number of C environments in a molecule and hence the number of signals in the ¹³C NMR spectrum

- Exact magnetic field seen by each nucleus in a molecule depends on:
 - The externally applied magnetic field
 - AND on the electronic environment surrounding the nucleus
- The extent to which electrons "shield" a nucleus depends on:
 - The bonding to that atom (single vs double vs triple)
 - The number of electrons associated with surrounding atoms (i.e. the types of atoms attached: C vs H vs O vs Cl vs N)



Carbon environments

- The number of signals in an NMR spectrum reflects the number of carbon environments in the molecule.
- Within a molecule two carbon environments are identical when the bonding and neighbours are identical.

What does a ¹³C NMR spectrum tell us?

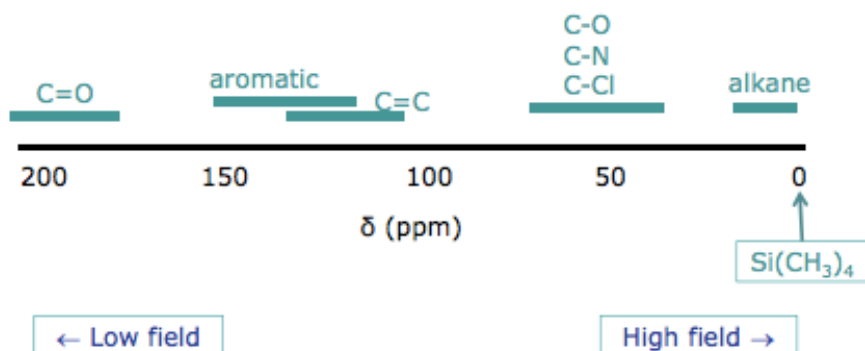
1. N^o of C environments \longleftrightarrow N^o of signals

2. Type of environment \longleftrightarrow Position of signal

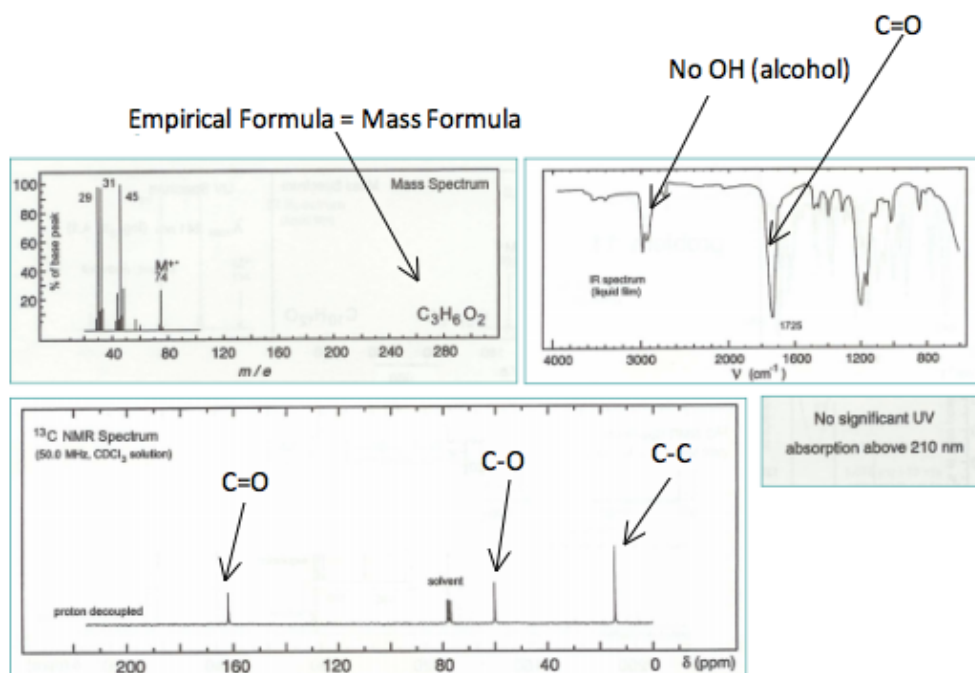
The position of a signal is called its chemical shift referenced to tetramethyl silane (TMS) ($\text{CH}_3)_4\text{Si}$ where TMS signal is set to $\delta = 0.00\text{ppm}$. (calibration)

Position of signal in spectrum correlates to nature of the carbon environment giving rise to that signal e.e. the types of groups/atoms and bonds (single, double or triple) attached:

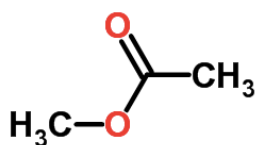
- Electronegative atoms (N, O, Cl) **de-shield** a C nucleus \rightarrow shifts signal downfield compared to an alkane (ie higher ppm than alkanes)
- Carbons of C=C or aromatic ring \rightarrow Shifted downfield
- Carbons of C=O \rightarrow Shifted furthest downfield



Determine the structure of a simple compound from the spectroscopic data



Molecule = Methyl Acetate = $\text{CH}_3\text{OCOCH}_3$



Function	Ppm on ^{13}C NMR spectrum
Alkanes	0- 30
Electromagnetic E.g C=O	50 - 70
C=C	80-110
C=C Aromatic Ring	110 - 150

**The less exposed to the magnetic field (i.e more stable) the higher the signal on graph which is why C=C in an aromatic ring has highest ppm.