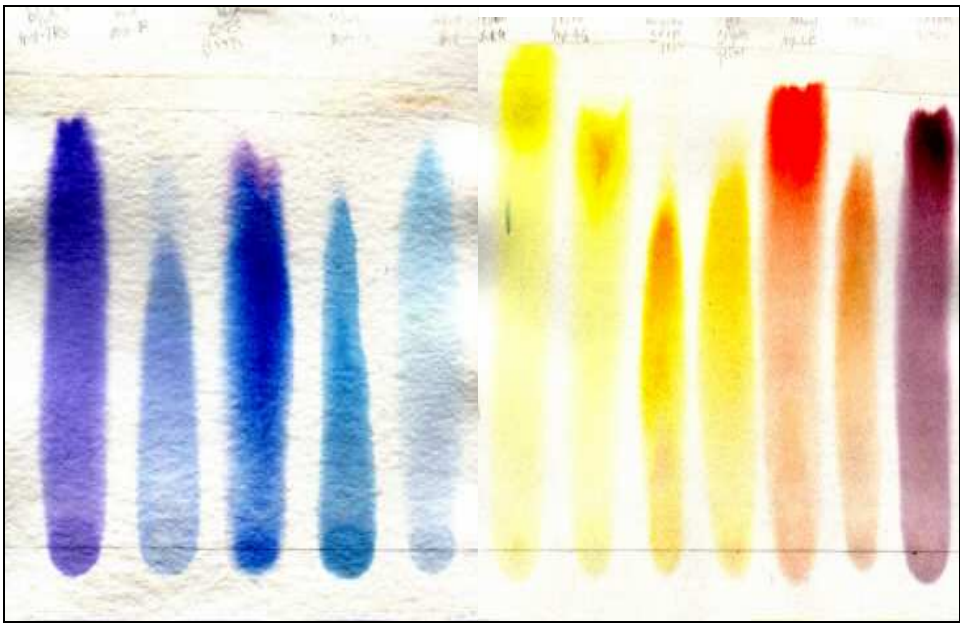
Name: _____

OCR A Chemistry

2815/04

Methods of Analysis and Detection



 Version 2.0

- Separation techniques of analysis (PC / TLC / GLC / Electrophoresis)
- Mass Spectroscopy
- Atomic emission spectroscopy
- UV / Visible absorption spectroscopy
- Combined spectral techniques (NMR / IR / Mass Spectra)

By U6F Jacky Huang 2008/03 ®

A. Introduction to chromatography

All chromatography have the following characteristics:

1. They all have 2 phases, one is a **stationary phase** and the other one is **mobile phase**.
2. The dissolved compounds – **solutes**.
3. There are 2 possible mechanisms, one is **partition** and the other one is **adsorption**.

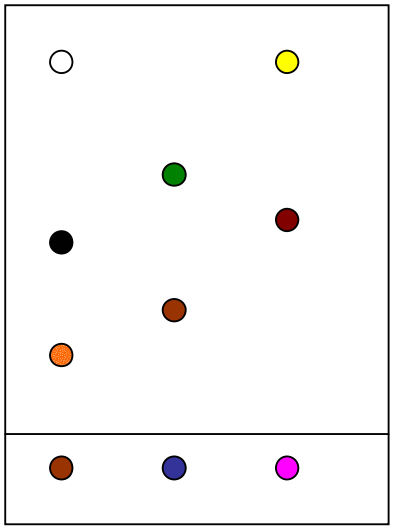
In partition:

The solutes move between the 2 phases. If it is in the mobile phase, the solutes will moves with it. Therefore, if we do spend more time in the mobile phase, it can move further.

In Adsorption:

The stationary phase is usually a polar solid and the solutes are polar molecules. The polar molecules will not enter the stationary phase, but it will hold on the surface of the polar stationary phase.

B. Paper Chromatography - The most basic chromatography



<-- Solvent Front
{
{
{
{
{Separated Material
{
{
{
{
<-- Reference Compound

Stationary Phase: **Cellulose fiber in the filter paper**
Mobile Phase: **Liquid Solvent**

In paper chromatography, coloured compounds can be separated into many colours, but in some cases, **colourless spots** are involved. When this happen, chemicals like **NINHYDRIN** will be sprayed to those substances, and those substances will form coloured complexes with these coloured compounds.

The solutes can be identified in 2 ways:

1. Comparison to the **reference compounds**
2. Calculation of **Retardation factor** =

$$\frac{\text{D moved by centre of solute spots}}{\text{D moved by front of mobile phase}}$$

Sometimes a **2-way chromatography** is carried out to ensure that all the solutions are fully separated.

C. Thin layer Chromatography – TLC- Simple, Cheap and Reliable

Stationary Phase: **Thin layer of silica**
Mobile Phase: **Liquid (Silica Gel)**

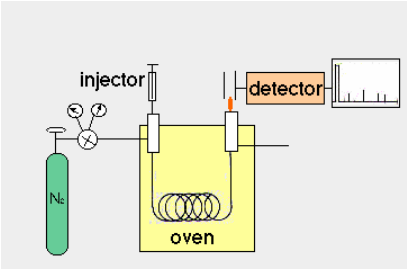
TLC is a very similar technique as paper chromatography, but it is 3 times **faster** than paper chromatography, works with **small amount** of sample, a **wider range** of mixtures separated.

In the stationary phase, the silica is heated to **remove water**, these acts as a polar solid and the solutes are transported by **adsorption** in mobile phase.
(When dry, both phases will be likely to occur)

However, silica will **attract water** and the presence of water becomes the stationary phase and the solutes are separated by **partition**.

Actual uses: **Clinical diagnosis / Forensic testing / Quality control**.

D. Gas/Liquid Chromatography - Use for very small sample



Stationary Phase: **THE SAMPLE itself**

Mobile Phase: **Inert Gases or carrier gases**

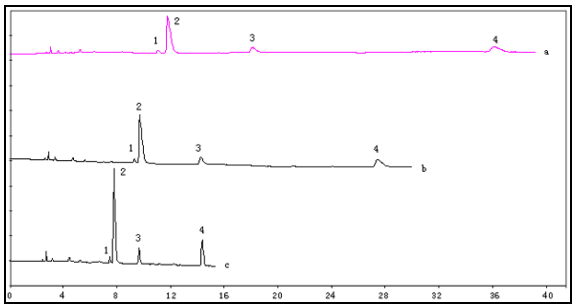
- ☑ GLC is worked by **partition**,
- ☑ Flows thru the column of stationary phase
- ☑ Separated due to different **solubility**,
- ☑ Or **volatility**,
- ☑ If the stationary phase is **non polar** → **Volatility.**
- ☑ Stationary is **polar** → polar molecules retain → Take **longer** to appear.

The sample will be detected by the detector and appear on **chromatogram.** Chromatogram tells us how much of a component is present, the area under the graph being related to the amount.

So how to **Determine the % composition of a mixture by GLC:**

The area of a peak = 1/2 (Base × Height)

Therefore, a chromatogram must show all the components and the detector are respond equally to all components.



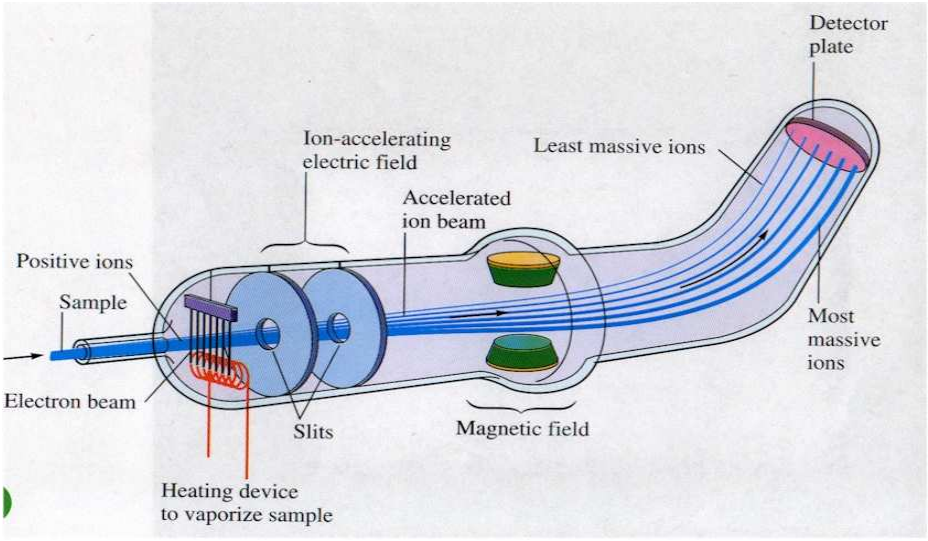
Green Tea Separation in GLC

【 Retention time: Time between injection and appearance of a component.】

The retention time is specific to particular components for the same conditions:

- ☑ **Same carrier gas**
- ☑ **Same flow rate**
- ☑ **Same stationary phase**
- ☑ **Same temperature**

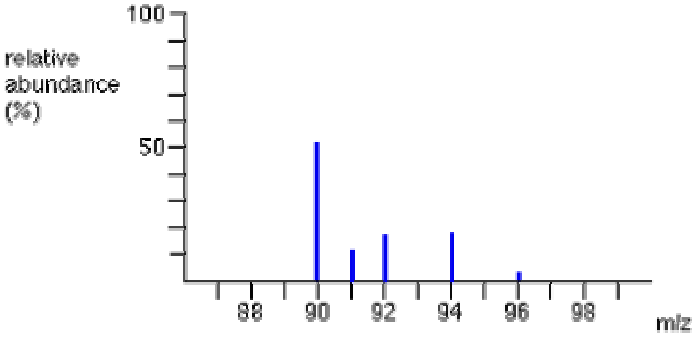
E. Mass Spectroscopy – A high resolution one (Double focusing)



In the detection of mass spectra, the following condition used in the experiment must be constant:

- ☑ The same Temperature
- ☑ The same ionizing voltage
- ☑ The same type of instrument

Analysis of an ion determine the A_r



The mass spectrum for zirconium

Number of isotope: **5** – 5 peaks can be observed.

Abundance of isotope:

Zirconium-90 51.5%

Zirconium-91 11.2 %

Zirconium-92 17.1 %

Zirconium-94 17.4 %

Zirconium-96 2.8 %

Relative atomic mass =

{90×0.515 + 91×0.112 + 92×0.171+ 94×0.174 + 96×0.028} / 100 = **91.3**

Analysis of compounds – Determine M_r and formulae

In high resolution mass spectra , we can examine the relative isotopic mass up to 4 d.p.

^{1}H	1.**0078**
^{14}N	14.**0031**
^{12}C	12.**0000**
^{16}O	15.**9949**

This will be given in the exam

So we will be able to distinguish 2 compounds have similar M_r by using mass spectra analysis.

E.g. M_r= 44 - Propene, C_3H_8, & ethanal, CH_3CHO

In a high resolution mass spectrometer, the molecular ion peaks for the two compounds give the following **m/z values up to 4 d.p**.

C_3H_8 44.**0624**

CH_3CHO 44.**0261**

M peak / M+1 peak / M+2 peak / M+4 peak

Peak	Original ions	Mr of isotope	Peak Height
M	Original Peak		
M+1	**Carbon -12**	**Carbon -13**	Too small
M+2	**Chlorine – 35**	**Chlorine – 37**	**3:1**
M+4	**Bromine - 79**	**Bromine - 81**	**1:1**

In mass spectra, we will be also to be able to work out the number of carbon inside a compound. (Values will be given in the exam)

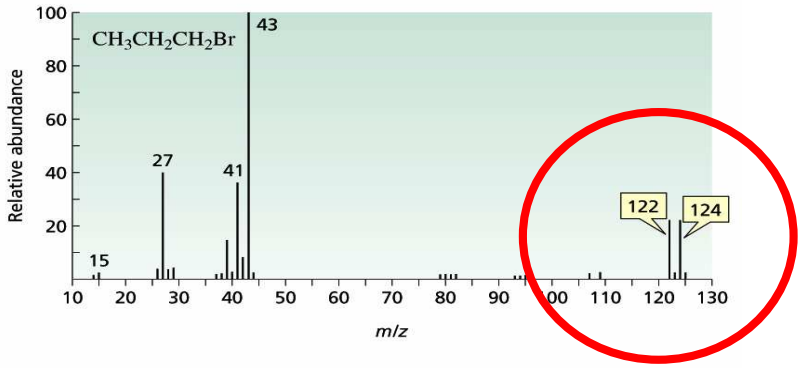
E.g. the M peak at m/e 112 has a height of 63.0mm and the M+1 peak at m/e 113 has a height of 4.1mm. **(1.1% that a ^{13}C will be presence)**

Ans: Height of (M+1) ÷ Height of (M) peak × 100

4.1mm / 63.0 mm × 100 = 6.5 %

6.5 / 1.1 = 5 = 5 carbons in the compounds

E.g. Mass Spectra for $CH_3CH_2CH_2Br$



m/z	Peak name	Responsible ions
122	M	**M Peak**
123	M+1	**M Peak** + **C – 13**
124	M+2	**M Peak** + **Br – 81**

Actual uses:

- ☑ **Traces of toxins** in food or water
- ☑ Testing **drugs on racehorse**
- ☑ **Cancer Treatment** – Urine analysis

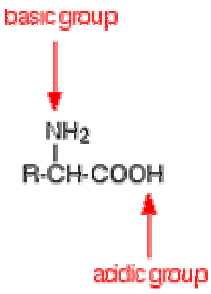
F. Electrophoresis – Biological analysis

Electrophoresis is separation of **charged particles by their movement in an electric field**. The visual presentation is by using – **Electropherogram.**

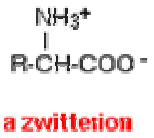
Although the **amino acid solution is colourless**, its position after a time can be found by spraying it with a solution of **ninhydrin**. If the paper is allowed to dry and then heated gently, a coloured spot will be observed.

In zone Electrophoresis, the mixture is a solution or gel,
The supporting medium is a filter paper made by cellulose,
Separated by the charge and the size of compounds.

A **highly charged ion** will **move faster** in an electric field.
A **larger ion moves slowly** across the supporting medium.

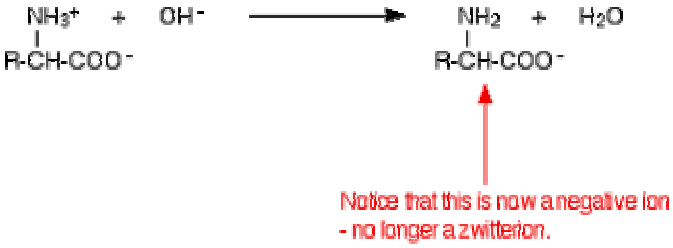


The electrophoresis need to be worked in a stable environment. The temperature and pH need to be controlled by adding buffer solution.

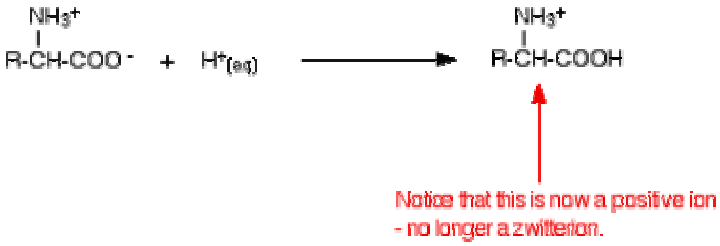


Normal amino acids will be look like the one on the left. If there is an internal transfer of a hydrogen ion, a **zwitterion** will be released.

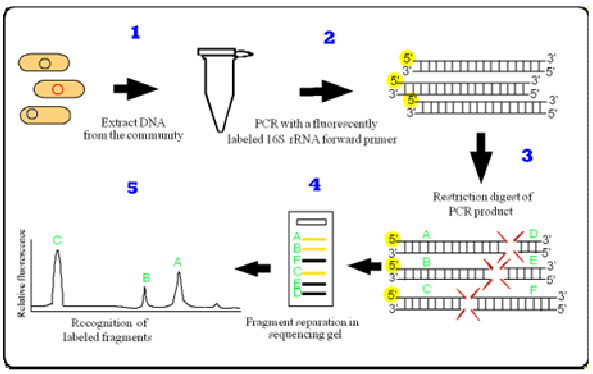
If the **pH has increased**, the zwitterions will then become,



If the **pH has decreased**, the zwitterions will then become,



One of the uses for electrophoresis will be used for genetic fingerprint:

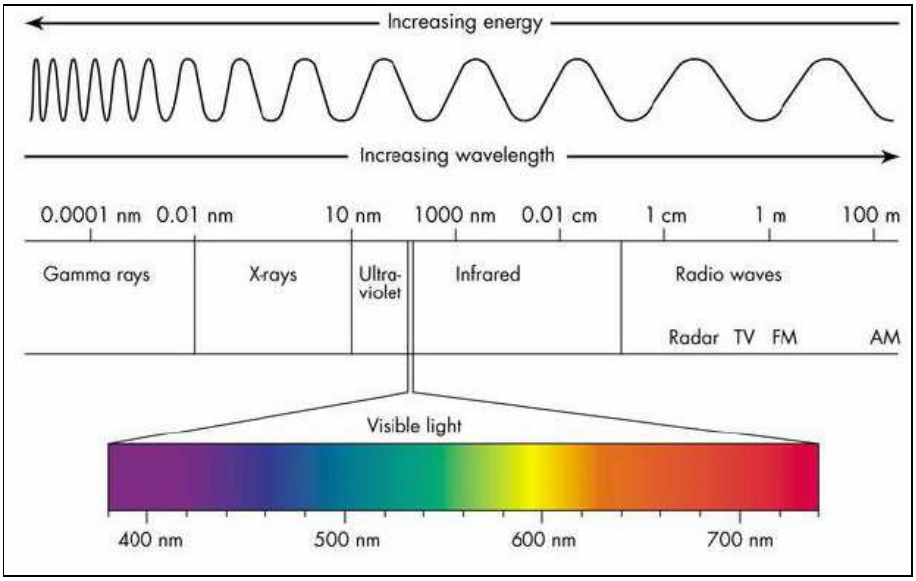


1. Take a DNA strand
2. Cut – **Restriction Enzyme**
3. Separated by **Electrophoresis**
4. Transferred to **Nylon membrane**
5. **P-32** DNA probes bind with DNA band
6. **X – Ray** exposed, Results.

There are also other uses for electrophoresis:

Forensic Science / GM Food / Medical Research / Establishing Relationships

G. Atomic Emission Spectroscopy – Interaction of EMR



This type of spectroscopy concerns the electromagnetic radiation with matter. The EMR have different frequency and different energy.
E.g. the **radio waves** change the orientation of **spinning nuclei** in a magnetic field and **infrared** cause's changes in **vibrational** energy.

Calculation for E= h f and C= f λ

If a molecule absorbs EMR of 356nm, calculat e the energy associated with EMR, directly to the energy gap between E_1 and E_2 or ΔE
In the Wave equation we know: **C = f λ**
C = 2.998 x 10^8 // f = frequency //λ = wavelength
2.998 x 10^8 = f x 3.56^7 → f = 8.42 x10^{14} **Hz.**

This means the energy associated with the EMR → **E =h f**
E = Energy // h = **Plancks' constant** = 6.63 x10^{-34} // f = frequency
E = 6.63 x 10^{-34} x 8.42 x 10^{14} = 5.58 x 10^{-19}**J**

If we want to calculate the energy for **one mole** we need to multiply the energy level by the **Avogadro constant** (6.023 x 10^{23}).
E = 5.58 x 10^{-19} x 6.023 x10^{23} = 336223.6J = **336kJ mol $^{-1}$**

Explain the Hydrogen absorption/emission spectrum

The molecular H (g) at a low pressure is **bombarded with electrons** in a discharging tube. The bombardment **dissociates the hydrogen molecules to give atom as well as excited atoms** so that they are **no longer in its ground state**. The excited atoms relax and the EMR seen as a pink glow.
This radiation is then passing thru a spectrometer which splits the EMR according to frequency.

Hydrogen Absorption Spectrum

☑ Black lines on continuous spectrum – EMR absorbed.

Hydrogen Emission Spectrum

☑ Discrete lines – EMR missing from the absorption spectra.

When the energy of the photons exactly matches the energy difference between the ground state and an excited state, **the radiation is absorbed by the atoms**. An atom in an excited state will rapidly – **relax** – this means it will revert back to its ground energy level. So the EMR used this characteristic to **let the atom to lose a photon.**

Calculate the ionization energy

For hydrogen the convergence limit is 3.30×10^{15} Hz, so we can calculate the IE by **E=h x f**:

$E = 6.63 \times 10^{-34} \times 3.30 \times 10^{15} \times 6.02 \times 10^{23}$

E = **1318 kJ mol^{-1}**

Quantitative Analysis of flame emission spectroscopy

This is a method that we used for finding how much metal ion is present in clinical diagnosis. E.g. determination of Na^+ in blood:

1. **Moisten a nichrome or platinum wire with conc. HCl,**
2. **Dip into the sample and place the wire to a Bunsen flame,**
3. **If Na^+ is present, a yellow / orange colour will be observed.**

H. UV/Visible absorption spectroscopy – changes in electronic structure

An Electromagnetic spectrum see section G on p.9

Here are 2 definitions that you need to know:

- ☑ **Chromophore** – are **unsaturated groups of atom** in organic molecules that **absorb radiation mainly in the UV/Vis regions**.
- ☑ **Conjugation** – the system of **alternate double bond and single bonds** in a molecule. **Organic molecules** with extended conjugation absorb radiation in the **visible part** of the spectrum and are therefore colored.

The wavelength at which organic molecules absorb radiation depends on how tightly their electrons are bound.

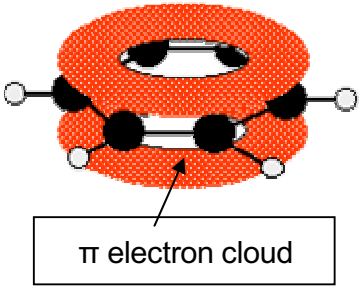
- ☑ Bonds – the shared electrons in **single bonds do not easily give absorption** spectra. But the electrons in **double and triple bonds are more loosely held** and easier to get excited.
- ☑ Lone pair – Lone pair that is **localized around atoms** (O_2 / N) is **loosely bound** and are **absorb in the same region** of the spectrum.

Ultraviolet and visible radiation are absorbed by the **outmost electrons** in organic molecules, which then move **from lower to higher energy** levels.
The outmost electrons can be:

- ☑ Electrons **directly involved** in **bond formation**,
- ☑ **Unshared** outer electrons.

『It is the movement of electrons between molecular orbital give rises to UV/Vis spectra.』

Some of the organic molecules have **colours**, because, they absorb certain frequencies of radiation **within the visible region**. (Wavelength 400 – 750 nm)
The **frequencies of radiation** absorbed by various chromophores can be used for the identification.



The wavelengths and the intensities of the absorption bands due to **chromophores are altered by the conjugation** in molecules and **delocalized electrons**.
In benzene, the electrons are evenly distributed a round the rings.

The conjugation of chromophores in a molecule causing **π orbital** interact with each other then become **delocalized** → Formation of **new orbital** → Energy **gap reduced** → absorption bands **shift to longer wavelength** → **intensity increase**.

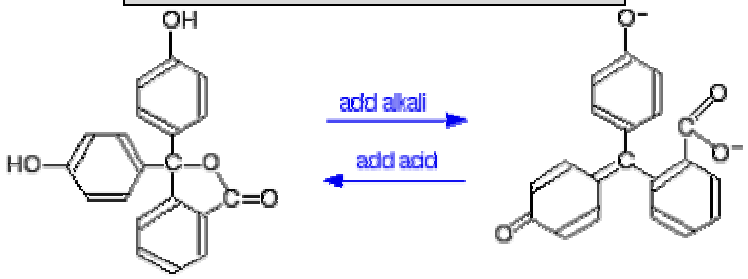
Effects of additional Chromophores will shift to an organic molecule more from the UV region to the visible region. See table below for more details.

Number of CH=CH	Region of Electronic transition	Colour
1 / 2	**UV**	**Colourless**
3	**Visible**	**Yellow**
15	**Visible**	**Dark Green**

Dyes and colour change in acid – base indicators

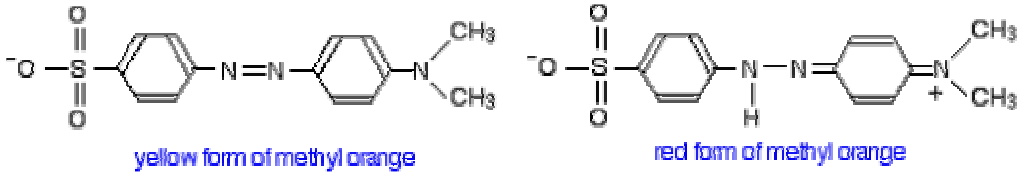
1. Phenolphthalein

Acidic = **Colourless** // Alkaline = **Red**

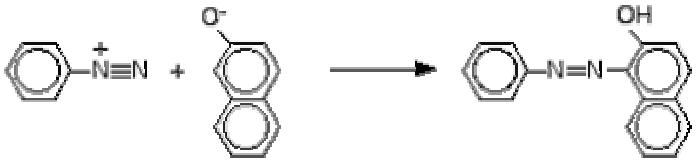


2. Methyl Orange

Acidic = **Red** // Alkaline = **Yellow**



3. Azo Dyes – coloured due to presence of chromophores



Benzene Diazonium + Napthalene-2-ol → **Red azo dyes** + HCl

I. Combined spectral Techniques – Analysis of mixture and compound

Now, we will be able to use evidence from N.M.R. / I.R. / Mass Spectra to suggest a probable structure for an unknown compound.

The following questions are from the OCR text book – methods of analysis and detection.

See page 50 -53 in OCR Chemistry – Methods of Analysis and Detection.

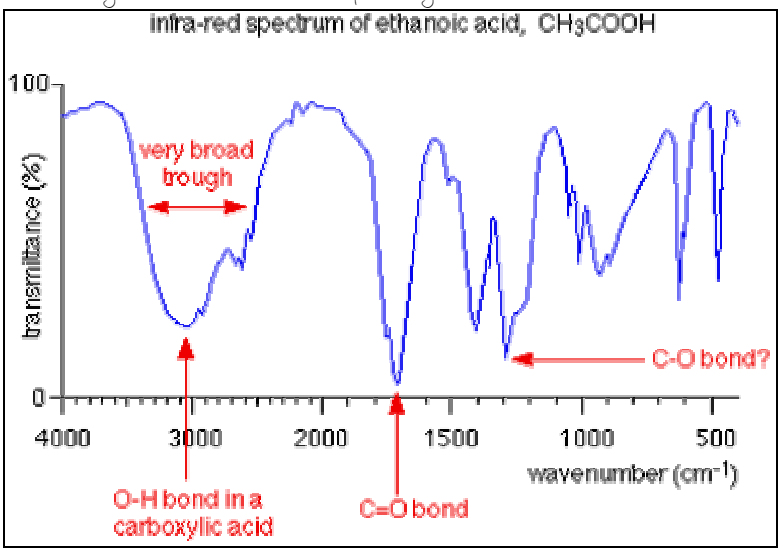
J. Infrared Spectroscopy (IR) – using wave numbers

Here, IR used the characteristics that different bonds in compounds **absorb different frequencies** in the infrared region of the spectrum. In infr ared analysis the wave numbers are used instead of frequencies or wavelengths.

In IR, note the following:

Compound	Things to note
Alcohol	Peak at 3400 (OH)
Carboxylic acid	Peak at 2500-3500 (**Broad** for OH) Peak at 1700 (C=O) Peak at 1000 -1250 (C-O)
Carbonyl Compounds	Peak at 1700 (C=O)
Ester	Peak at 1700 (C=O)

Example of an IR spectrum – Ethanoic Acid



K. Nuclear Magnetic Resonance Spectroscopy (NMR) –Splitting Pattern

The peak in the NMR gives many information of compound; this is a summary in a table of what it tells you:

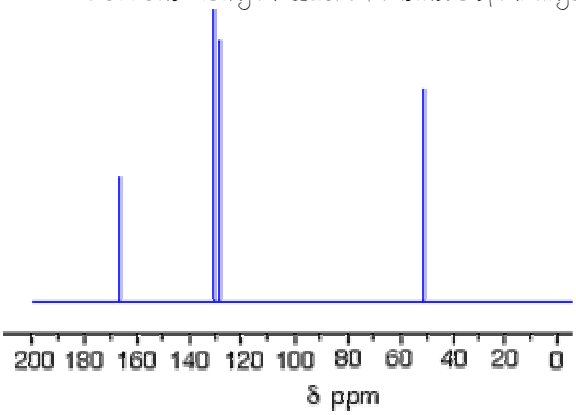
What it tells you	How this is shown in the spectrum
How **many types** of proton present	The **number of distant peak** = number of proton
How **many of each type** of proton	The **relative peak area** under the peak = relative number of proton
What **type** of proton	The **value of ξ** and ten use the table provided in the exam
The number of chemically different protons **adjacent to a particular type of proton**	By the splitting of the distant peak. If there is n chemically different type of protons next to a particular type of proton then the peak for that proton is split to **n+1** peaks

Use D_2O with –OH group

Deuterium is an isotope of hydrogen that does not produce an NMR peak.

If an alcohol or carboxylic acid is shaken with **D_2O**, **the H of the OH group is replaced by D** and the **peak disappears** from the spectrum.

Example of a NMR spectrum – $C_4H_6O_2$



1. 4 peaks and 4 Cs, **2 of them are in the same environment.**

2. The peak just **over 50 must be C-O**

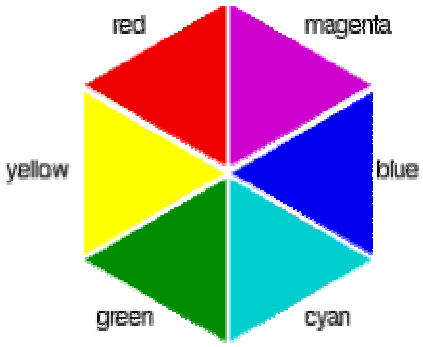
3. 2 peaks around 130 must be 2 carbons at either end of a **C=C**.

4. The peak at just less then 170 is **the C in a C=O**.

5. By using IR and Mass Spectroscopy, we can work out the structural formula.

L. Additional Notes and Materials

Colour wheel can be used to determine a compounds colour:



Colours	nm
Red	625-740
Yellow	565-590
Green	520-565
Cyan	500-520
Blue	435-500
Magneta	380-435

Colours directly opposite each other on the colour wheel are said to be complementary colours. Blue and yellow are complementary colours; red and cyan are complementary; and so are green and magenta.

E.g. Beta-carotene absorbs throughout the ultra -violet region into the violet - but particularly strongly in the visible region between about 400 and 500 nm with a **peak about 470 nm** and if we use the table above, we know it will absorb **blue colour** so the colour we will see will between **red and yellow**.

M. Bibliography and Reference

1. http://www.chemguide .co.uk
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6. Sherborne School resources
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