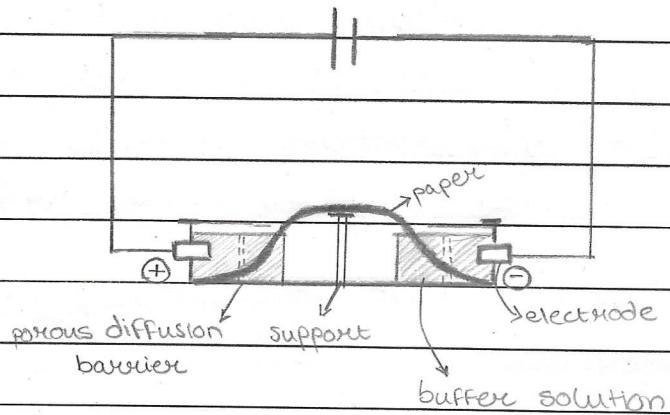




### Electrophoresis : (paper)



## 29- Applications of analytical chemistry

### Q-1) Electrophoresis.

- > Electrophoresis is based on separating ions placed in an electric field.

The sample is placed on absorbent paper or a gel supported on a solid base. A buffer solution carries the ions along.

The rate at which they move depends on:

- size ; small ions move quickly
- charge ; highly charged ions move quickly

- \* Depending on the side groups, amino acids have tendencies to form either cations (+ve) or anions (-ve), depending on the pH. Proteins are usually treated to make them negatively charged so they all move towards the anode with varying speeds.

### Q-2) Paper chromatography (partition)

- > Paper chromatography is used to separate mixture as a solvent moves up a piece of absorbent paper.

**stationary phase:** water trapped between cellulose fibres of paper

**mobile phase :** Solvent eg: ethanol, acetone.

The substances in the mixture have different affinities for the solvent and the water ∵ will move at different rates.

$$\text{Retardation factor (R}_f\text{)} = \frac{x}{y} = \frac{\text{distance moved by solute spot}}{\text{distance moved by solvent}}$$

Solvent front

The RF values are then compared with reference values obtained under identical conditions.

\* High RF value = more affinity in mobile phase.

\* Colourless substances are sprayed with a chemical that forms coloured compounds on the chromatogram.

eg: amino acids are revealed as bluish spots by ninhydrin spray.

### Two-way chromatography

Sometimes components in a mixture can have similar RF values in a particular solvent  $\therefore$  are difficult to separate.

Two-way chromatography is used:

- ① Paper chromatography is carried out normally
- ② Paper is rotated  $90^\circ$
- ③ chromatography is again carried out with a different solvent.

\* Partition: separation due to different solubilities of compounds in the 2 phases.

\* Adsorption: separation due to different attractions between the compounds and the stationary phase, relative to their solubility in the solvent.

Q-3) Thin-layer chromatography (TLC) (adsorption)

**stationary phase:** solid which adsorbs solute molecules onto its surface

eg: alumina ( $\text{Al}_2\text{O}_3$ ), silica ( $\text{SiO}_2$ ) on glass plate.

**mobile phase:** ~~polar~~ solvent.

**Advantages of TLC over paper chromatography:**

- ① TLC is faster
- ② TLC can be used on smaller samples

\* polar molecules have greater affinity for polar stationary phase.

Q-4) High performance liquid chromatography (HPLC) (partition)

**stationary phase:** non-volatile liquid

eg: long chain hydrocarbon bonded on solid support [eg: silica ( $\text{SiO}_2$ )]

**mobile phase:** polar solvent eg: methanol, water

- > The detector records retention times i.e. how long it takes for each component to pass through the column.
- > Area under each peak is proportional to amount of solute emerging from the column.

**Advantages:**

- not only identifies compounds but also gives relative proportions of each compound in the mixture.



Q-5)

### Gas - Liquid chromatography (partition)

**stationary phase :** non-volatile liquid on a solid support

eg: alkane (with high BP) coated on SiO<sub>2</sub>.

**mobile phase :** inert gas eg: Nitrogen.

- > Volatile components will spend more time in the mobile phase and will have a shorter retention time.

$$\% \text{ composition of A} = \frac{\text{Area of A peak}}{\text{Sum of areas of A, B, C... peaks}}$$

Uses :

- testing for steroids in athletes
- analysing blood samples
- testing fuel in racing cars
- detecting pesticide residues in foods.

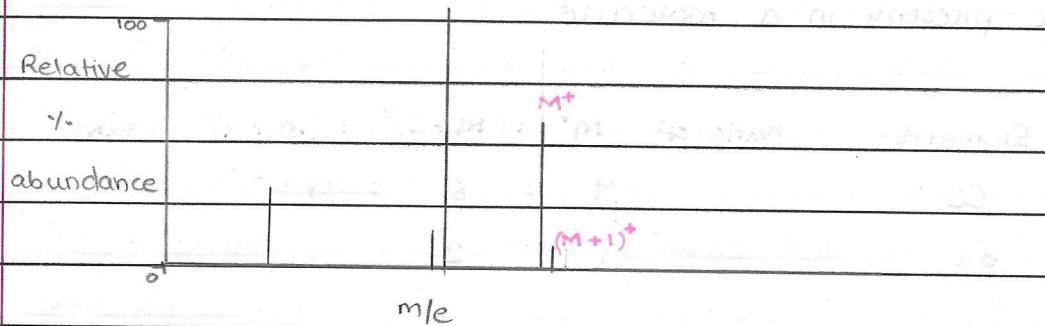
Q-6)

What is retention time?

- > It's the time taken for a component to travel between injection and detection.



## Q-7) Mass spectrometry



- > The peaks are caused by the fragmentation of the molecule into positive ions.
- > The  $M^+$  peak is the molecular ion peak.
- > The  $(M+1)^+$  peak is due to the presence of  $^{13}C$  isotope.

$$\text{no. of carbon atoms (n)} = \frac{100}{\% \text{ abundance of } M^+ \text{ ion}} \times \frac{\% \text{ abundance of } (M+1)^+ \text{ ion}}{\% \text{ abundance of } M^+ \text{ ion}}$$

- >  $(M+2)^+$  peaks occur due to the presence of  $^{35}\text{Cl}$  and  $^{37}\text{Cl}$ , and  $^{79}\text{Br}$  and  $^{81}\text{Br}$  isotopes.

**Element      Ratio of  $M^+$  :  $(M+2)^+$  peak.**

Cl	3 : 1	$M$	$M+2$

Br	1 : 1	$M$	$M+2$



- >  $(M+4)^+$  peak can also occur if two Cl or Br atoms are present in a molecule.

Element      Ratio of  $M^+ : (M+2)^+ : (M+4)^+$  peaks.

Cl                9 : 6 : 1

Br                1 : 2 : 1

### Q-8) Proton ( $^1H$ ) NMR spectroscopy.

- > The nucleus of a hydrogen atom consists of a single proton which has a SPIN (odd atoms have a spin)

When an external magnetic field is applied, the protons either spin with the direction of the field, or against the direction of the field. (less stable).

When the proton flips to the higher energy level (against direction of magnetic field), it absorbs energy in the RF region.

Each hydrogen atom needs different energy, depending on its environment.

In NMR, the chemical shift values are measured relative TMS (tetramethylsilane) which is at 0 ppm.

This is because:

① All 12 Hydrogens are in same environment

② C-H bonds are closer ∴ H's are most shielded ∴ most energy required to flip the protons.

This results in a single peak on the extreme right-hand side on the NMR spectra.

### Low Resolution NMR

- Number of peaks gives no. of different environments
- Area under peak gives no. of  $^1\text{H}$  atoms.

### High resolution NMR.

> The spinning nuclei interfere with the neighbouring nuclei which results in spin-spin coupling.

This causes the peaks to split into  $n+1$  peaks.

$n$  = no. of  $^1\text{H}$  atoms on adjacent carbon.

Relative intensities in splitting

0	1 peak	↪ singlet
1	2 peaks	↪ doublet
2	3 peaks	↪ triplet
3	4 peaks	↪ quartet

### Analysing NMR spectra:

- Use chemical shift values to identify environment of each proton.
- Look at area under peak to deduce no. of H atoms in each environment.
- Use  $n+1$  rule to find no. of H atoms on adjacent carbons.
- Join all the above information and deduce the compound.



- > NMR are usually measured using solutions of the substance being investigated.  
It's important that the solvent doesn't contain H-atoms as this would cause peaks in the spectrum.  
For this, Deuterium solvents (eg  $\text{CDCl}_3$ ) are used. Deuterium has different magnetic properties than hydrogen ∵ doesn't produce peaks in the region that we're looking at in the NMR spectrum.

- > The -OH and -NH peaks are not split in NMR as the protons ( $\text{H}$ ) rapidly replaces with protons ( $\text{H}$ ) in traces of water.

These peaks can be identified by:

- adding  $\text{D}_2\text{O}$  (heavy water).

The Deuterium ( ${}^2\text{H}$ ) rapidly exchanges with the  ${}^1\text{H}$  and the -OH or -NH peak disappears from the spectrum.

\* NMR only works with atoms with odd mass no.

### Q-9)

#### Carbon-13 NMR spectroscopy

- > About 1% of the carbon atoms are  ${}^{13}\text{C}$ , the rest are  ${}^{12}\text{C}$ . The C-13 produces an NMR spectrum, similar to the hydrogen/proton NMR.
- > The solvent used is  $\text{CDCl}_3$ . This causes a small peak at 80 ppm and can be ignored.
- > chemical shift values are measured relative to TMS.
- \* no. of peaks represents no. of different environments of  ${}^{13}\text{C}$ .
- \* the peaks don't split in  ${}^{13}\text{C}$ -NMR.