3A - Separation

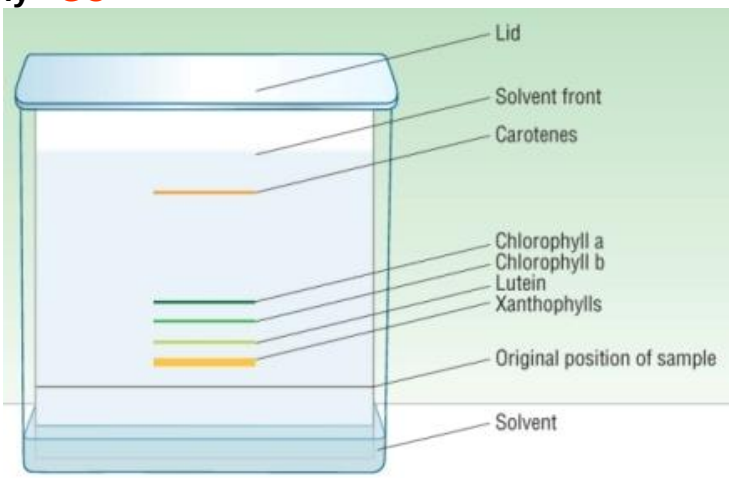
Separation by chromatography

What is chromatography?

- Chromatography is a separation technique first used to separate pigments in plant leaves.
- There are 2 types of chromatography that you will study:

1) Thin Layer Chromatography - TLC

2) Gas Chromatography - GC



- The plate is placed in a tank with the solvent below the sample.
- The tank is covered and allowed to rise near the top of the plate.
- The plate is removed, the solvent front marked and the solvent allowed to evaporate.
- The data is analysed.
- Chromatography is used in analysis but is more widely used as a method of separation.

How does chromatography work?

- Chromatography works on the basis that components (molecules) have a different affinities for a **stationary phase** and for a **mobile phase**

Phases:

A) Stationary phase - is in a fixed place (paper in paper chromatography)

- The molecules interact with the stationary phase slowing down their movement - **ADSORPTION**

B) Mobile phase - moved in a definite direction (water rises up in paper chromatography)

- The molecules interact with the mobile phase speeding up their movement - **SOLUBILITY**

OVERALL - DIFFERENT MOLECULES MOVE AT DIFFERENT SPEEDS DUE TO THEIR DIFFERING INTERACTIONS WITH THE 2 PHASES

1) Thin Layer Chromatography - TLC

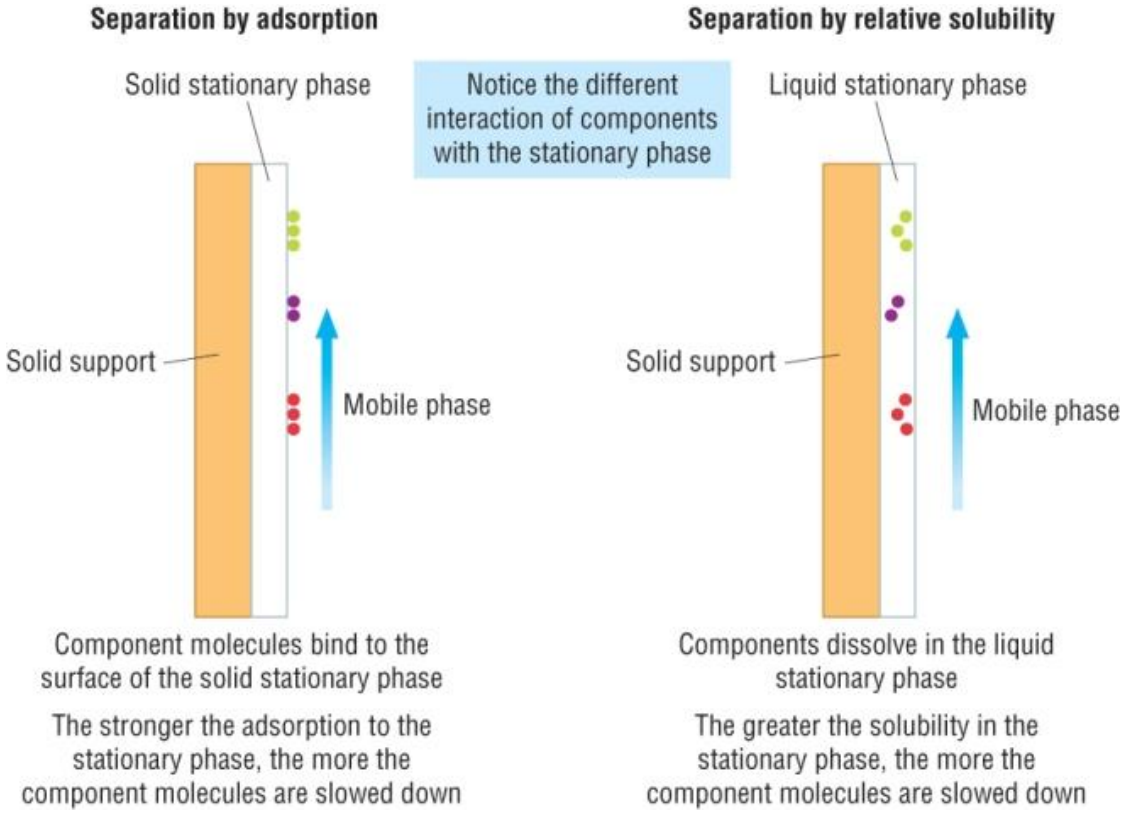
- Solid stationary phase and liquid mobile phase

2) Gas Chromatography - GC

- Liquid on solid support stationary phase and a gas mobile phase

Separation:

- Is by adsorption or solubility depending on whether the stationary phase is a liquid or a solid:



Qu 1,2 P77

<u>Thin - Layer Chromatography - TLC</u>

- Is used to check purity / monitor the extent of a reaction.

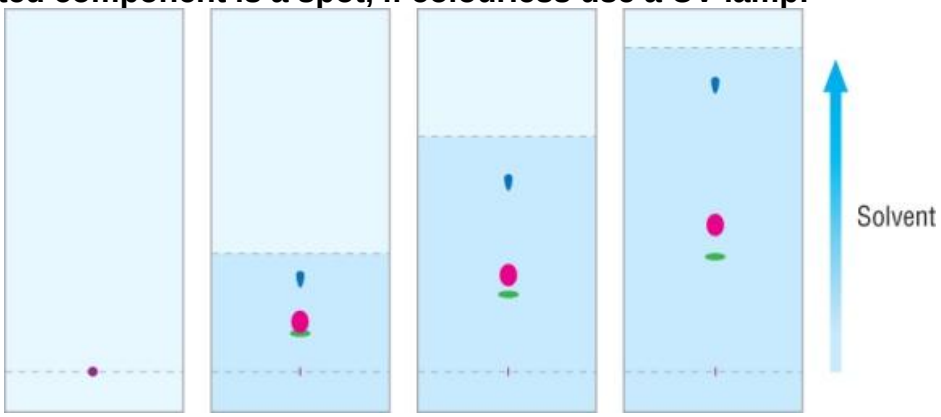
Phases:

A) Stationary phase - Silica gel, SiO_2 or Aluminium oxide, Al_2O_3

B) Mobile phase - Solvent

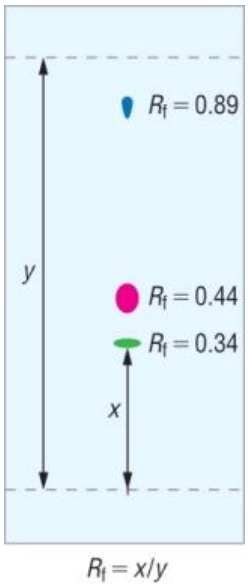
Producing the chromatogram:

1) Dissolve sample.
2) Draw a pencil line and spot sample using a capillary tube, allow to dry.
3) Place plate in a tank of solvent - solvent must be below line, seal the tank.
4) Separation is by adsorption - allow solvent to almost reach the top, draw a line here - solvent front.
5) Each separated component is a spot, if colourless use a UV lamp:



R_f values:

- An R_f value shows how far a component has travelled compared with the solvent front:



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

$$R_f \text{ Green} = \frac{1.65}{4.85} = 0.34$$

$$R_f \text{ Pink} = \frac{2.15}{4.85} = 0.44$$

$$\frac{4.30}{4.85}$$

$$R_f \text{ Blue} = 0.89$$

- R_f is a ratio of the distance of the component moved : solvent front.
- This means that the R_f values in this solvent will always be the same:

For substances that are very soluble in the liquid, R_f will be close to 1

For substances that are not very soluble in the liquid, R_f will be close to 0

- Comparisons can also be made against pure components.

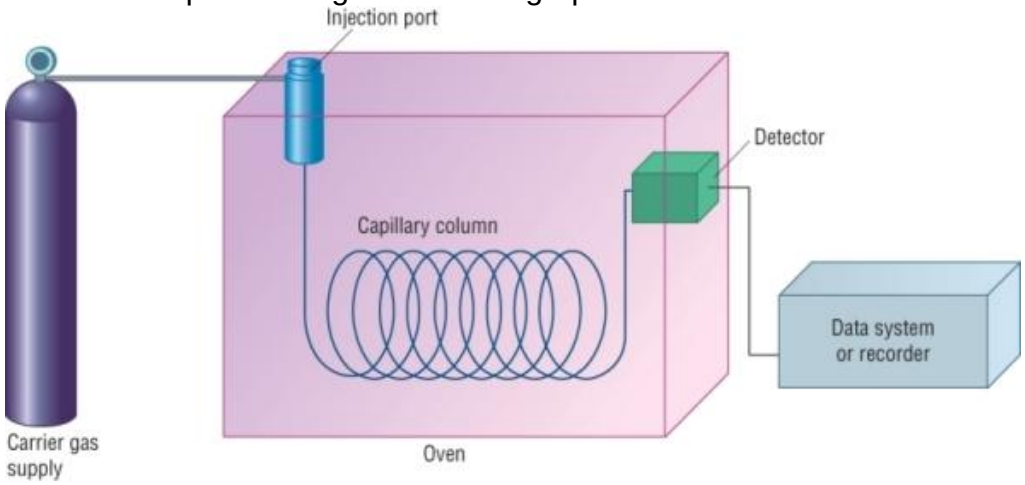
Limitations:

- Similar compounds often have too similar R_f values.
- Unknown compounds have no R_f value for comparison.
- It is hard to find a solvent that will have the carrect amount of solubility - Goldilocks!!

Qu 1,2 P79

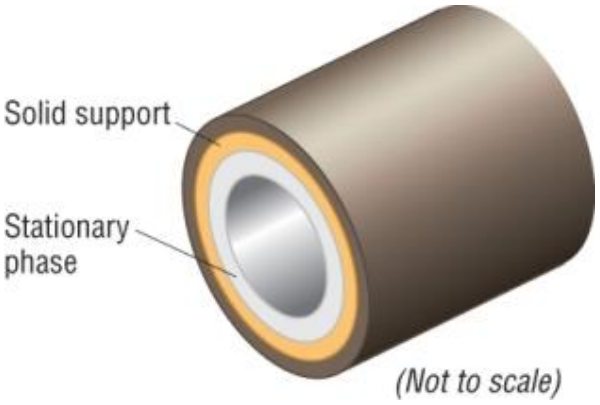
Gas Chromatography - GC

- Is used to separate volatile compounds (gases) in a mixture.
- The compounds, being volatile will have low boiling points (to evaporate easily
- This process takes place in a gas chromatograph:



Producing the chromatograph:

- The mixture is injected into the chromatograph, it is vaporised and the mixture is carried through the column by the mobile carrier gas.
- As the mixture flows through the capillary column, the components are slowed down by the stationary phase lining the column:



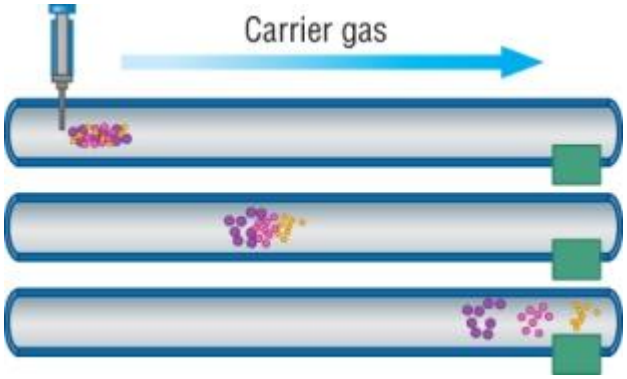
The stationary phase:

- The **stationary phase** is a liquid or solid lining of the capillary tube.
- This tubing is called the chromatography column.
- A suitable **liquid** lining for the **stationary phase** is usually a long chain alkane (high boiling point) - **solubility**
- A suitable **solid** lining for the **stationary phase** is usually a silicone polymer - **dsorption**
- Depending on what is separated depends on whether you use a liquid or solid stationary phase.

The mobile phase:

- Is an inert carrier gas such as helium or nitrogen.

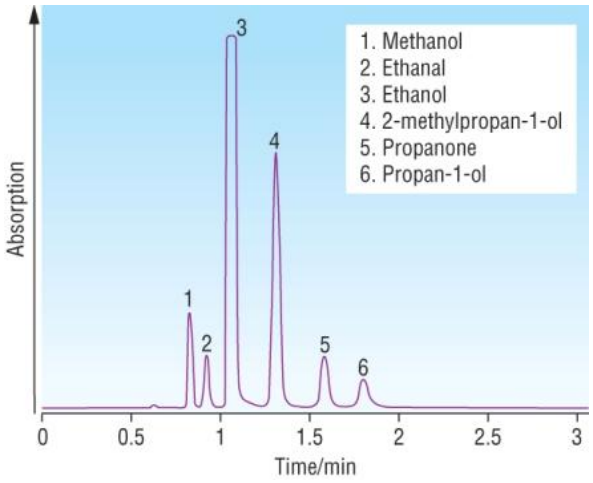
- Different components are slowed down by different amounts - **separation:**



- Separation can be improved by using different flow rates and oven temperatures.
- Each component leaves the column at a different time and is detected as it leaves the column.
- The time taken for a component to leave the column is called the **retention time**:

Retention time:

- Is the time taken for a component to pass from inlet to detector.
- Just like R_f values the retention time can be used to identify a component.
- Known compounds will have known retention times at the same temperature, carrier gas and stationary phase.
- The area under each peak (component) is equivalent to the amount of that component in the sample:



- This is the gas chromatogram of blood / alcohol.
- The relative concentrations of ach component can be estimated by comparing peak areas.

Limitations of gas chromatography:

- Thousands of chemicals have similar retention times, peak shapes. This means that most compounds cannot be positively identified.
- Not all substances can be separated. Some substances can 'hide' under others. This can give a higher concentration of the other.
- Unknown compounds have no reference retention times. Analysts need to know what is expected.
- Due to the limitations, gas chromatography is usually used in conjunction with spectroscopy.

Qu 1,2 P81

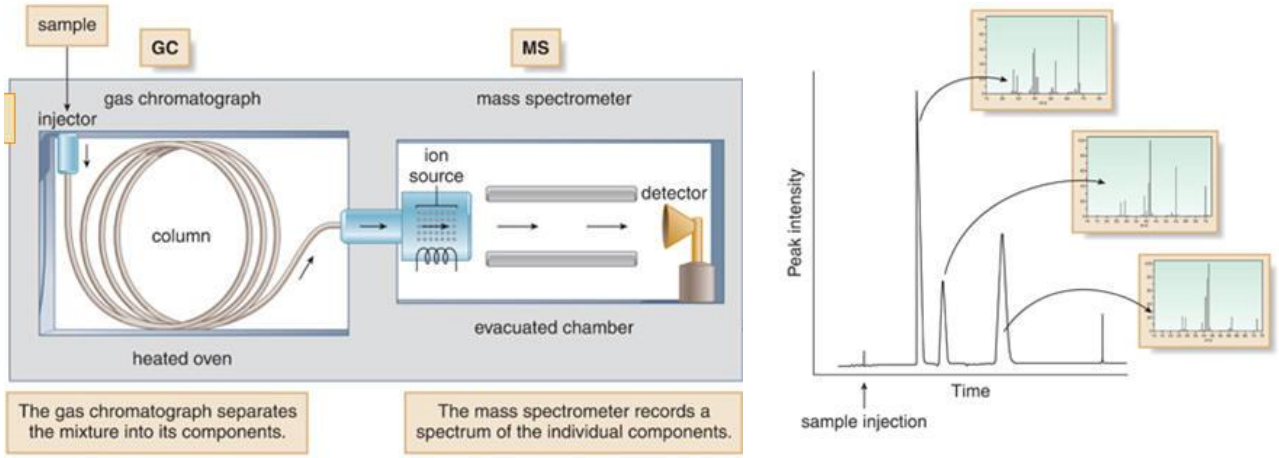
<u>Gas Chromatography - Mass Spectroscopy - GC-MS</u>

Combining gas chromatography with mass spectroscopy:

- This is 2 techniques combined to provide a powerful analysis tool.

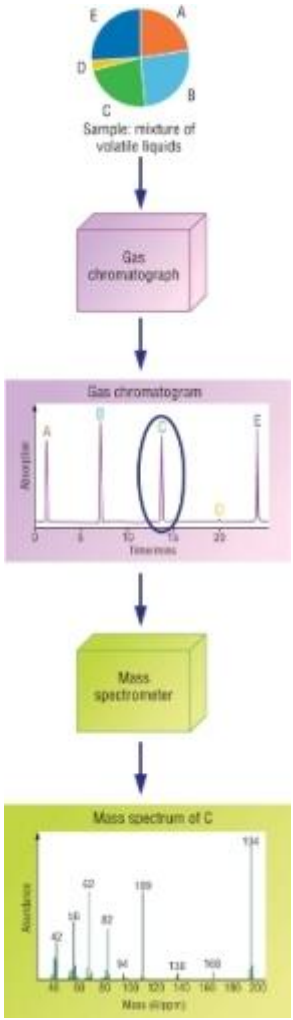
Gas Chromatography, GC — **Separates components**

Mass Spectroscopy, MS — **Gives detailed structural information**



- Each component is separated using GC then analysed using MS.

Summary:



Uses for GC-MS

1) Forensics - scenes of crime

2) Environmental analysis - air pollutants, waste water, pesticides in food.

3) Airport security - explosives in luggage / airport security

4) Space probes - planetary atmospheres

Qu 1-3 P83 Qu 1,2 P105 Qu 1 P107