3.16 Chromatography

Chromatography is an analytical technique that separates components in a mixture between a mobile phase and a stationary phase.

The mobile phase may be a liquid or a gas. The stationary phase may be a solid (as in thin-layer chromatography, TLC) or either a liquid or solid on a solid support (as in gas chromatography, GC)

Types of chromatography include:

- thin-layer chromatography (TLC) – a plate is coated with a solid and a solvent moves up the plate
- column chromatography (CC) – a column is packed with a solid and a solvent moves down the column
- gas chromatography (GC) – a column is packed with a solid or with a solid coated by a liquid, and a gas is passed through the column under pressure at high temperature.

In gas-liquid chromatography GC the **mobile** phase is a inert **gas** such as nitrogen, helium, argon.
The **stationary** phase is a **liquid** on an inert solid.

Separation by column chromatography depends on the balance between solubility in the moving phase and retention in the stationary phase.

If the stationary phase was polar and the moving phase was non- polar e.g. hexane. Then non-polar compounds would pass through the column more quickly than polar compounds as they would have a greater solubility in the non-polar moving phase. (Think about intermolecular forces)

A solid stationary phase separates by adsorption,
A liquid stationary phase separates by relative solubility

Retention times and Rf values are used to identify different substances.

Method: **Thin-layer chromatography**

a) **Wearing gloves**, draw a **pencil line** 1 cm above the bottom of a TLC plate and mark spots for each sample, equally spaced along line.
b) Use a capillary tube to add a **tiny drop** of each solution to a different spot and allow the plate to air dry.
c) Add solvent to a chamber or large beaker with a lid so that is no more than **1cm in depth**
d) Place the TLC plate into the chamber, **making sure that the level of the solvent is below the pencil line**. Replace the **lid to get a tight** seal.
e) When the level of the solvent **reaches about 1 cm from the top of the plate**, remove the plate and mark the solvent level with a pencil. Allow the plate to **dry in the fume cupboard.**
f) Place the plate under a **UV lamp** in order to see the spots. Draw around them lightly in pencil.
g) Calculate the Rf values of the observed spots.

Wear plastic gloves to prevent contamination from the hands to the plate

pencil line –will not dissolve in the solvent

tiny drop – too big a drop will cause different spots to merge

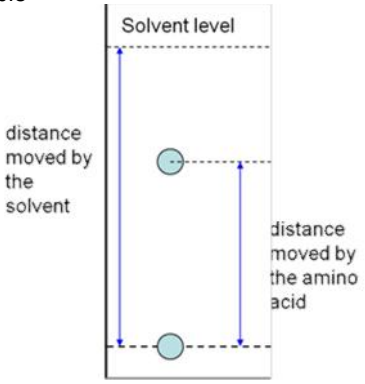
Depth of solvent– if the solvent is too deep it will dissolve the sample spots from the plate

lid– to prevent evaporation of toxic solvent

Will get more accurate results if the solvent is allowed to rise to near the top of the plate but the Rf value can be calculated if the solvent front does not reach the top of the plate

dry in **a fume** cupboard as the solvent is toxic

UV lamp used if the spots are colourless and not visible

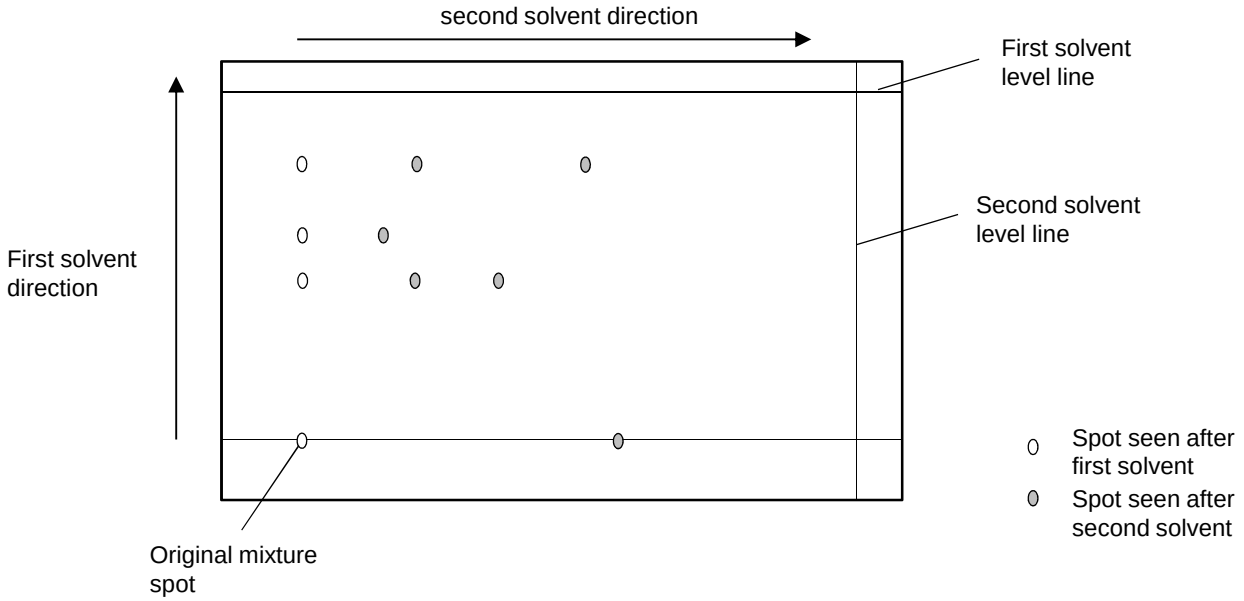


R_f value = $\frac{\text{distance moved by amino acid}}{\text{distance moved by the solvent}}$

Two directional chromatography

In order to separate a complex mixture that has components of different solubility in solvents, it may be necessary to do chromatography with two different solvents.

A spot of the mixture on a TLC plate is first separated with one solvent.
Then the TLC plate is rotated 90° and the plate is placed in a second solvent for a second separation to take place



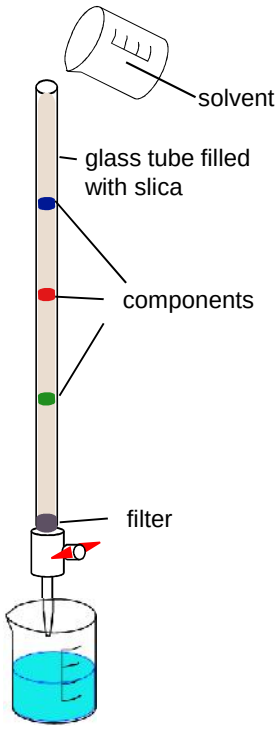
In total this mixture has 6 different components.

This process would be done if components in the mixture have the same Rf value or if some components are not soluble in the first solvent

Column chromatography (CC)

Simple column chromatography

- A glass tube is filled with the stationary phase usually silica or alumina in powder form to increase the surface area.
- A filter or plug is used to retain the solid in the tube. Solvent is added to cover all the powder.
- The mixture to be analysed is dissolved in a minimum of a solvent and added to the column.
- A solvent or mixture of solvents is then run through the column.
- The time for each component in the mixture to reach the end of the column is recorded (retention time)



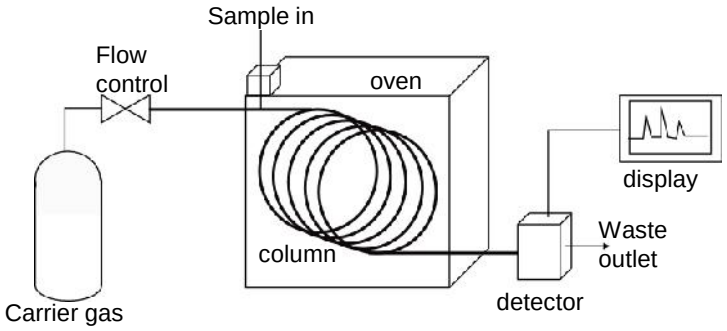
HPLC stands for high performance liquid chromatography and it type of column chromatography commonly used in industry.
HPLC: **stationary** phase is a **solid** silica
HPLC: **mobile** phase a **liquid**

Gas-Liquid Chromatography

Gas-liquid chromatography can be used to separate mixtures of volatile liquids.

In gas-liquid chromatography, the mobile phase is an inert gas such as helium and the stationary phase is a high boiling point liquid absorbed onto a solid.

The time taken for a particular compound to travel from the injection of the sample to where it leaves the column to the detector is known as its **retention time.** This can be used to identify a substance.



Some compounds have similar retention times so will not be distinguished.

Basic gas-liquid chromatography will tell us how many components there are in the mixture by the number of peaks. It will also tell us the abundance of each substance. The area under each peak will be proportional to the abundance of that component.

It is important to use an inert carrier gas such as helium or nitrogen. These will not react with the components being separated in the GC column.

The following factors can be changed to change the retention times of substances being separated: GC column temperature, column length , flow rate.
If the temperature or the flow rate is higher then substance will move more quickly through the column to give shorter retention times.

It is also possible for gas-liquid chromatography machine to be connected to a mass spectrometer, IR or NMR machine, enabling all the components in a mixture to be identified.

Most commonly a mass spectrometer is combined with GC to generate a mass spectra which can be analysed or compared with a spectral database by computer for positive identification of each component in the mixture.

GC-MS is used in analysis, in forensics, environmental analysis, airport security and space probes.