6.3.1 Chromatography and Analysis

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)

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

TLC Chromatography (thin-layer chromatography)

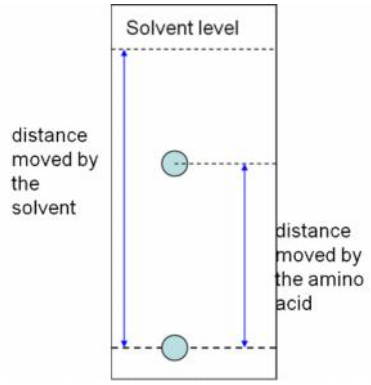
A mixture can be separated by chromatography and identified from the amount they have moved. (Can be used with mixtures of amino acids)

R_f value = distance moved by amino acid / distance moved by the solvent

Each substance has its own R_f value

Measure how far each spot travels relative to the solvent front and calculate the *R*f value.
Compare *R*f values to those for known substances.

Method
Take chromatography paper and draw a pencil line 1.5cm from bottom.
With a capillary tube put a small drop of amino acid on pencil line
Roll up paper and stand it in a large beaker.
The solvent in the beaker should be below the pencil line.
Allow to stand for 20 mins and mark final solvent level
Spray paper with ninhydrin and put in oven



Some substances won't separate because similar compounds have similar *R*f values. So some spots may contain more than one compound

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

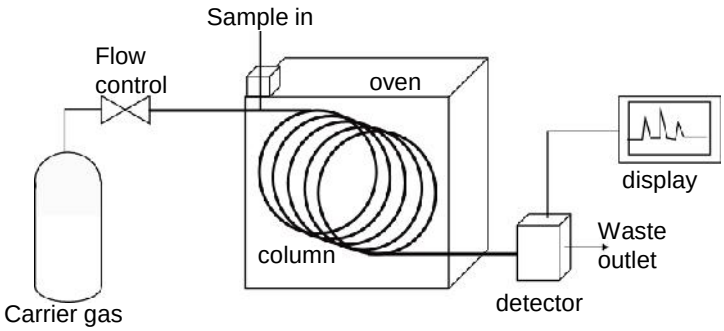
UV lamp used if the spots are colourless and not visible

Gas-Liquid Chromatography

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

In gas-liquid chromatography, the mobile phase is a 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

Callibration

To calculate the concentration of each component in the curve it is necessary to complete external calibration curves to confirm concentrations of components. Known amounts of a pure component can be passed through the GC machine. The calibration curve will give the retention time of the component and the area under the curve (the peak integration value) will be a measure of the pure concentration. This can then be compared with the retention times and integration values of the components in the mixture to work out the amounts and proportions of the components in a mixture.

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

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.

Testing for functional groups

Functional group	Reagent	Result
Alkene	Bromine water	Orange colour decolourises
carbonyl	2,4-DNP	Orange precipitate formed
Aldehyde	Tollens' Reagent	Silver mirror formed
Carboxylic acid	Carbonate ions CO_3^{2-} e.g. Sodium carbonate	Effervescence of CO_2 evolved
1^o 2^o alcohol and aldehyde	Sodium dichromate and sulphuric acid	Orange to green colour change
haloalkane	Warm with aqueous silver nitrate in ethanol	Slow formation of white precipitate
phenols	Will react with sodium and sodium hydroxide- won't react with Carbonate ions CO_3^{2-}	Fizzing with sodium but no reaction with sodium carbonate