Chromatography

Chromatography separates individual substances from a mixture.

- to find out how many components there are
- to match the components with known reference materials
- to use additional analytical techniques to identify what each component is

In any form of chromatography separation occurs because there are two **phases**: a **mobile phase**, which sweeps the mixture in a definite direction over a **stationary phase** which is fixed in position, and which slows down each compound in the mixture by a different amount, leading to separation.

Thin Layer Chromatography (TLC)

The **mobile phase** dissolves the components in the mixture and moves vertically up the TLC plate. A **liquid** solvent is used – it must not react with the components in the mixture. The solvent will need to be similar to the substances to be identified so they will dissolve.

As they move over up the TLC plate, the components in the mixture are adsorbed on the surface of the **stationary phase** then desorbed again continually, slowing down their movement up the TLC plate. The stationary phase is **solid** - a thin layer of e.g. alumina (Al_2O_3) or silica gel (SiO_2) coated on a flat inert support, usually a sheet of glass or plastic.

- The more strongly a substance is adsorbed, the more time it will spend on the stationary phase, and the more slowly it will move.
- The more soluble a substance is in the mobile phase, the more easily it will be desorbed and are swept along more quickly.

Definition: **ADSORBED** = weakly bonded to a surface

Identification

A chromatogram of the unknown mixture is often run alongside samples of known pure substances (references) thought to be in the mixture. Matches can be made to components that travel the same distance up the TLC plate.

The relative distance a spot travels compared to the solvent is called the **retention factor**, R_f. This can be used to identify a component by comparing with the published R_f values of pure substance (measured under the same conditions with the same solvent).

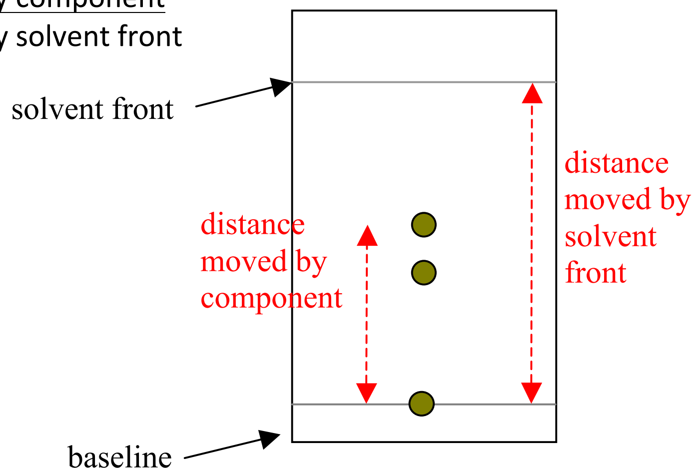
Method

- spot of the dissolved sample is dotted on the pencil baseline
- spots of pure samples that may be present in the mixture may be dotted alongside
- spots allowed to dry, then TLC plate placed in jar with shallow layer of solvent

- solvent allowed to rise near top (max separation) then position of solvent front marked
- positions of spots examined – if colourless, a locating agent (such as ninhydrin) or UV light may be used to show up each spot
- chromatogram interpreted, matching spots and using R_f values to quantify positions

Determining R_f values

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



Practice:

A baseline is drawn 10mm from the bottom of a TLC plate and a mixture containing components A and B spotted. After elution with a suitable solvent, the solvent front was 100mm from the bottom of the TLC plate.

i) Component A has an R_f of 0.3. Where was this spot now located
ii) Component B was 55mm from the bottom of the TLC plate. Calculate its R_f.

Limitations

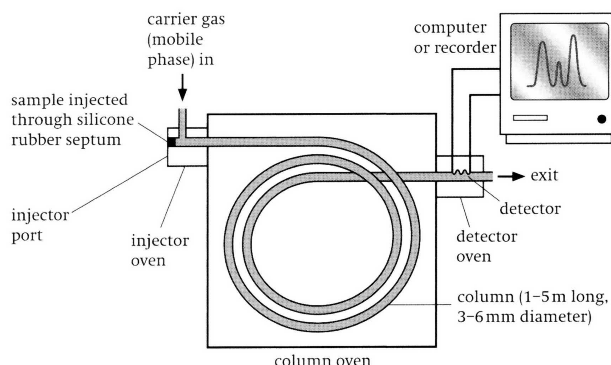
- chemically similar compounds may have similar R_f values
- may tell you how many components are present, and R_f values may suggest what they may be, but DOES NOT give information about how much of each component is present.

Gas Chromatography (GC)

Very commonly used instrumental technique, often fully automated. Used to separate **VOLATILE** components in a mixture (gases, liquids, occasionally volatile solids).

Basic components

- carrier gas supply
- injection port
- chromatography column
- detector and computer/data system/recorder



The **chromatography column** is a capillary tube. It is very long (up to 30m) and wound as a coil to fit inside a temperature-controlled oven.

In GC the **stationary phase** is a **liquid** coated on the surface of the capillary tube, in which the components in the mixture dissolve and then evaporate, slowing down their movement through the column. The liquid needs to be inert and non-volatile (high boiling point), so a long chain alkane is often used.

The **mobile phase** is a **carrier gas** which flows through the column carrying the sample mixture over the stationary phase. It must be inert e.g. He or Ne.

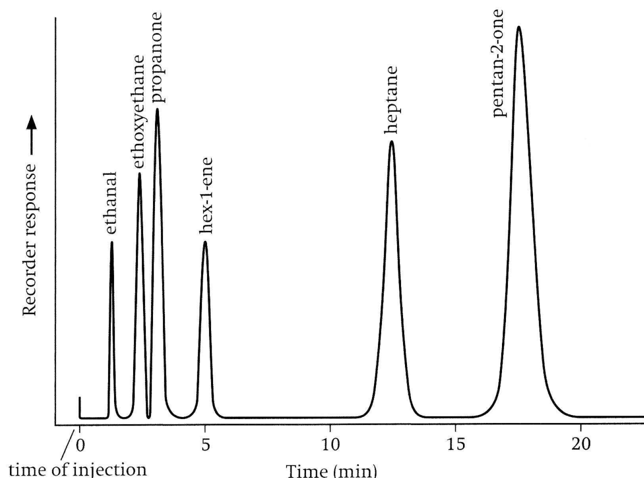
How it works

Differences in **solubility** of each component in the liquid forming the stationary phase cause separation. The more soluble each component is in the liquid stationary phase, the more time it will spend dissolved and the more slowly it will travel through the capillary. The time each component takes **from injection to detection**, is called its **retention time**. Each component has a characteristic retention time (for given stationary phase, temperature, gas flow rate etc.) that can be compared to published values for pure substances to aid identification.

Quantitative GC

The detector signal shows a peak for each substance as it emerges from the capillary. The area under each peak is proportional to the amount of that compound in the sample, so GC provides the **relative** amounts of each component in the mixture.

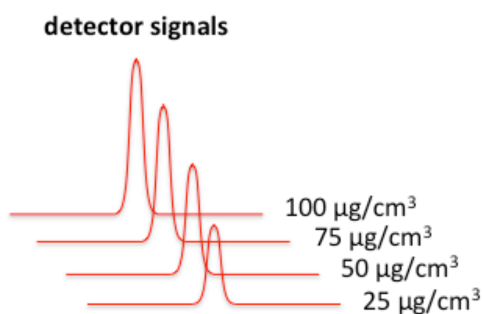
e.g.

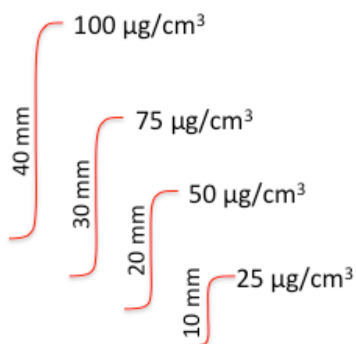


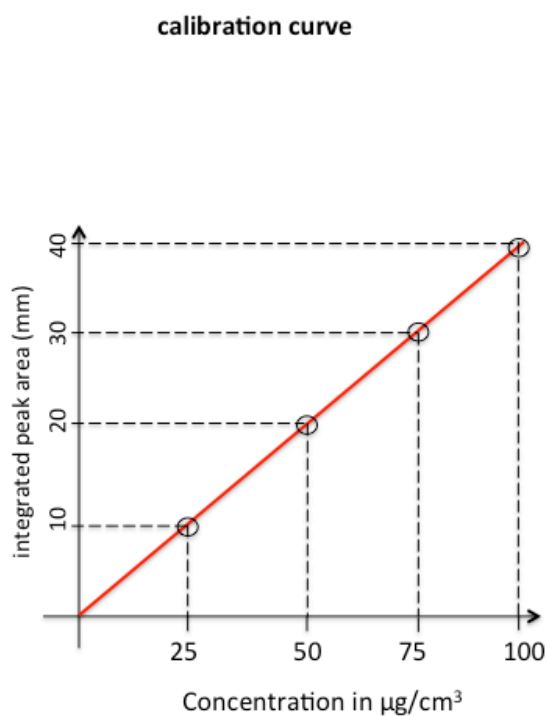
An integration trace can be provided to allow easy measurement of peak areas: the computer integrates each peak, and the height of the resulting step is proportional to the peak area.

External Calibration

A series of **standard solutions** containing a pure substance thought to be in the mixture can be prepared, each with a different concentration. These solutions can be used analysed using the GC instrument under the same conditions as will be used to analyse the unknown mixture, and graph plotted of known concentration (x-axis) vs. measured peak area (y-axis) – a straight line fit would be expected. Such a graph is called a **calibration curve**, and allows the peak area for that component in the unknown sample to be read off as a concentration of the component in the sample mixture.







Limitations of GC

- potentially thousands of substances may have same retention time, peak shape etc. so GC does not positively identify components
- not all substances in the sample will necessarily be separated and detected – small peaks can be hidden under larger ones

Advantages over TLC

- more rapid
- amounts of each substance can be measured
- smaller quantities of materials may be detected

Qualitative Analysis for Organic Functional Groups

Functional Group	Chemical Test	Observations
Alkene	Add a few drops of bromine water	Bromine decolourised from orange
Haloalkane	Add silver nitrate solution and ethanol, warm to 50°C in water bath	Chloroalkane – white precipitate Bromoalkane – cream precipitate Iodoalkane – yellow precipitate
Carbonyl (aldehyde, ketone)	Add 2,4-dinitrophenylhydrazine	Yellow/orange precipitate
Aldehyde	Add Tollens reagent and warm	Silver mirror
Primary or Secondary Alcohol or Aldehyde	Add acidified potassium dichromate(VI) and warm in water bath	Colour change from orange to green
Carboxylic Acid	Add aqueous sodium carbonate	fizzing
Phenol	Test with pH paper and aqueous sodium carbonate. Confirm by adding bromine to aqueous solution of the phenol	Turns pH paper red, but does not fizz with sodium carbonate White precipitate, and bromine decolourised