chromatography

2 chromatography

Its a method for separating and identifying the chemicals in a mixture.

Chromatography can be used to:

1. to separate the components of a mixture

3 chromatography

2. to determine the identity of the components of a mixture (usually by comparison with known compounds).
3. to determine the amounts of the various components present in a mixture (usually by comparison with a standard).

4 mechanism of chromatography

All chromatographic techniques involve a stationary phase and a mobile phase.

The components in a mixture are separated because of their differences in affinity for the stationary and mobile phases.

5 mechanism of chromatography

There are two basic phenomena that may be exploited to bring about separation of the components of a mixture: adsorption and partition.

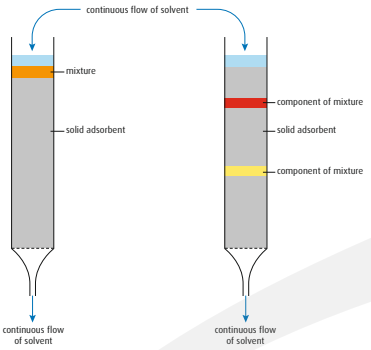
6 adsorption

Separation occurs by adsorption in column chromatography.

The column is packed with small particles of a solid (the stationary phase). The mixture is placed on the top of the column and a solvent (the mobile phase) is passed through the column.

7 adsorption

The components of the mixture separate as they move down the column.

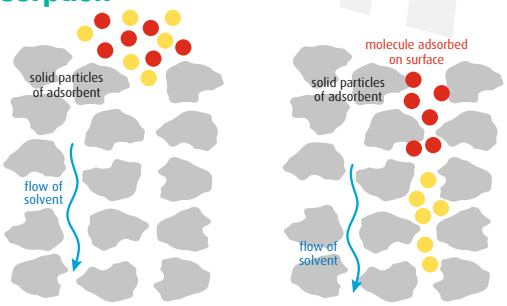


8 adsorption

The different components of the mixture separate from each other as they have different tendencies to be adsorbed onto the surface of the solid particles.

The more polar the particles, the greater the tendancy they will have to be adsorbed on the staionary phase.

9 adsorption



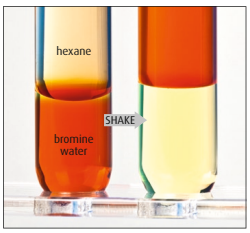
10 partition

Partition works because a solute will be distributed between two immiscible solvents according to its solubility in each solvent.

If an aqueous solution of bromine is shaken with hexane, most of the bromine moves into the hexane layer, as bromine is more soluble in hexane.

11 partition

In partition chromatography, the stationary phase is a liquid.



12 partition

Separation occurs because of the different tendencies of the components of the mixture to dissolve in the stationary phase solvent or in the mobile phase solvent.

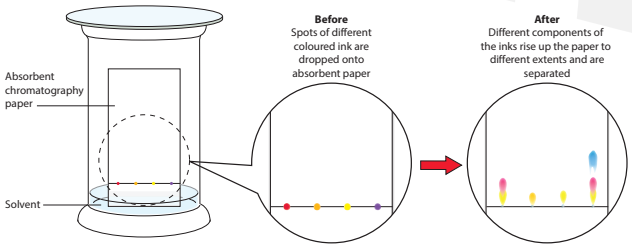
A substance that is more soluble in the stationary phase will travel more slowly through the system.

13 paper chromatography

In simple paper chromatography, a mixture of coloured substances is spotted onto a sheet of absorbent paper.

The paper dips into a solvent and the solvent separates the components of the mixture.

14 paper chromatography

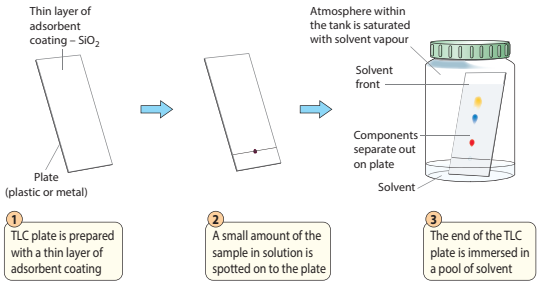


15 paper chromatography

This is a simple and quick way of finding out the components of a mixture of coloured substances (chromatography literally means 'colour writing').

There are several types of chromatography – they do not all involve paper, or even coloured substances.

16 thin layer chromatography (TLC)



17 thin layer chromatography (TLC)

The adsorbent solid on the plate is a very fine powder, giving a high surface area for adsorption.

The adsorbent solid on the plate is often a thin layer of silicon(iv) oxide (silica, SiO_2).

18 thin layer chromatography (TLC)

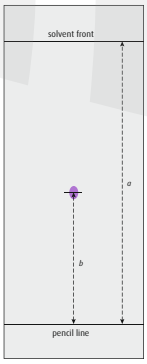
As the solvent rises up the plate, the components get partitioned between the solid silica and the liquid solvent.

Different components travel different distances.

19 R_f

We can describe the position of spots on a paper chromatogram or tlc using the retardation factor (R_f) value:

$$R_f = \frac{\text{distance solute moves}}{\text{distance solvent front moves}}$$

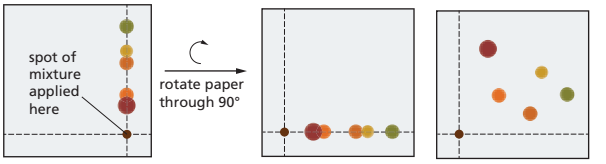


20 R_f

An R_f value close to 1 indicates that a component has a very high affinity for the mobile phase,

whereas a very low R_f value means that the component hardly moves at all from the base line and has a very high affinity for the stationary phase.

21 two dimensional chromatography



after running in first solvent (two pairs of overlapping spots)

before running in second solvent

after running in second solvent (all spots now separated from each other)

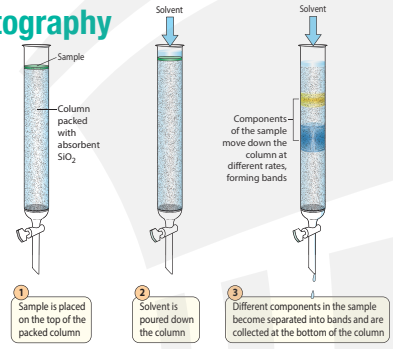
22 paper vs tlc

Paper chromatography	
method of separation	partition
stationary phase	water on the fibres of the paper
mobile phase	the solvent

Thin-layer chromatography	
method of separation	adsorption
stationary phase	solid particles (silica/ silica gel or alumina) coating the plate
mobile phase	the solvent

23 column chromatography

The mobile phase moves down by gravity and carries the components of the sample, which travel at various speeds and leave the column at different times.



24 column chromatography

The various components of the mixture are adsorbed to different extents on the stationary phase (solid particles of silica gel).

The component with the greatest tendency to be adsorbed on the stationary phase travels most slowly through the column.

25 column chromatography

The separated components are detected as they come out of the column at different times.

The time each component spends in the column (the retention time) can be used to compare unknown components with a known reference substance.

26 detector

The detector records retention times,

The area under each peak recorded is proportional to the amount of solute emerging from the column.

27 hplc

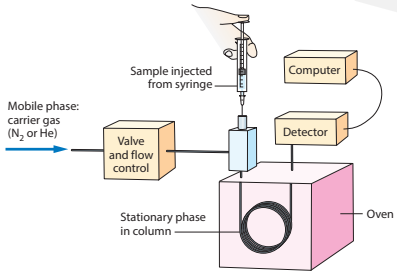
Various modifications of column chromatography include high-performance liquid chromatography (HPLC) that uses a solid stationary phase and a liquid mobile phase pushed through the column at high pressure.

28 gas chromatography (GC or GLC)

And gas chromatography (GC) with a gaseous mobile phase and a liquid .

GC is primarily used for the identification of volatile compounds in environmental, medical, and forensic studies.

29 glc



30 glc

In GC, the mobile phase is a gas, not a liquid.

The inert carrier gas, usually nitrogen or helium, flows through a column inside a heated oven. The inside of the column is coated with a thin layer of a stable, non-volatile liquid, such as silicone oil.

31 glc

The sample being analysed could be a gas or a volatile liquid, which vaporises in the oven. When it is injected into the gas stream, the sample gets carried along through the column.

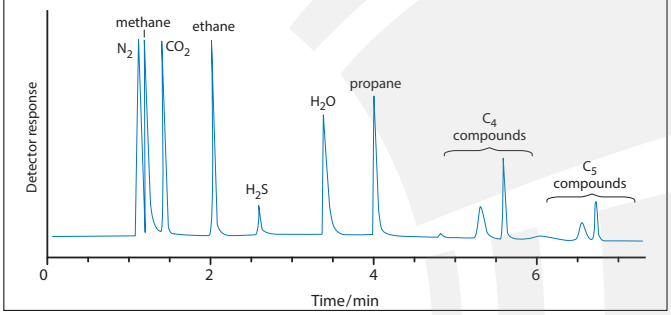
The components are partitioned between the gas phase and the liquid in the column.

32 glc

Components that are absorbed most strongly by the liquid stay longer in the column and come out last.

Components that are adsorbed least strongly by the liquid come out first. The time that a component spends in the column is its retention time.

33 glc



34 glc

the above shows gas chromatogram for a sample of natural gas from a refinery.

Each peak represents a different compound.

The height/area of each peak is approximately proportional to the relative quantity of the component in the mixture.

35 glc

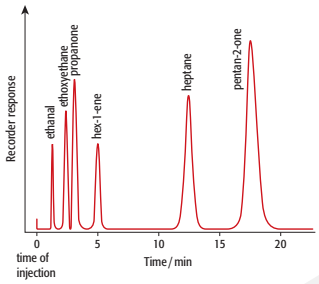
Gases like methane and N_2, with small molecules, have low retention times and come out of the column early. Gases with larger molecules have longer retention times.

36 lc vs glc

Column chromatography	
method of separation	adsorption
stationary phase	solid particles of silica gel or alumina
mobile phase	the solvent
movement of mobile phase	gravity

Gas–liquid chromatography	
method of separation	partition
stationary phase	high boiling point liquids (e.g. polysiloxanes) on a solid support
mobile phase	the carrier gas – He or N_2
movement of mobile phase	carrier gas flows through the system

37 glc



38 types of chromatography

Type	Stationary phase	Mobile phase	Used for
Paper chromatography	Paper	Solvent rising up the paper	Separating mixtures to identify their components
Thin layer chromatography (TLC)	An adsorbent solid spread thinly on a metal or plastic plate	Solvent rising up the plate	Separating mixtures to identify their components

39 types of chromatography

Type	Stationary phase	Mobile phase	Used for
Column chromatography	An adsorbent solid packed in a column	Solvent poured down the column	Separating mixtures to identify their components. Using the separated components for further chemical reactions
High performance liquid chromatography (HPLC)	An adsorbent solid packed in a column	Solvent forced through the column under pressure	Separating mixtures to identify their components. Using the separated components for further chemical reactions
Gas–liquid chromatography (GLC)	Organic liquid spread on surface of an unreactive column, heated in an oven	Unreactive gas forced through the column under pressure	Separating mixtures of gases or volatile liquids to identify their components