

CHROMATOGRAPHY

Chromatography is for components of a mixture. All types of chromatography have a stationary phase, which is a substance in a fixed position, and another substance that moves over it called the mobile phase.

Thin-layer chromatography has a stationary phase which is a thin layer of silica spread thinly over a glass or plastic plate and dried onto it.

A spot of sample is placed on the TLC plate near one end. The plate is placed with that end just dipping into a solvent, which is the mobile phase, and which slowly soaks up into a thin-layer.

Separation occurs by the movement of components of the sample onto the surface of the thin-layer. Components which are less strongly adsorbed move upwards on the plate more quickly or further.

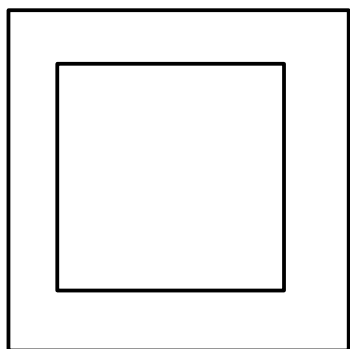
A lid is usually placed on the TLC chamber to stop the solvent from evaporating.

A standard is often used in TLC for easy comparison.

The chromatogram is complete when the solvent front gets close to the top of the plate.

The number of separated components is equal to the number of spots which can be seen. If the components are coloured they are easily seen, colourless components, such as amino acids can be located by spraying with a suitable chemical, such as ninhydrin.

The R_f value (retardation factor) of each component can be found by:



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

It may be possible to identify components using a range of R_f values. If different components are close to each other, they are likely to have similar R_f values so they may not be distinguished and cannot be distinguished. Compounds with similar structures such as alcohols are very difficult to distinguish for this reason.

Gas chromatography (GC) is used to separate mixtures of more components. It uses a long-coiled tube inside an oven. The inside surface of the tubes is usually coated with an oily, viscous liquid (usually an alkane). This is the stationary phase. The mobile phase is an unreactive or inert gas such as nitrogen.

A mixture is injected into the tube near the one end, and different components take different times to pass through to the other end. This depends on their volatility in the viscous stationary phase. This is sometimes referred to as the retention time. The more volatile they are, the more time they take to pass through the tube.

The retention time is the time taken for a component of a mixture to pass through the gas chromatography column.

Sometimes the stationary phase is a solid the method of separation would then be called gas-solid chromatography.

Gas chromatography has similar limitations to TLC as similar compounds will have similar retention times. Therefore, it may be difficult to distinguish them.

There is a detector that records the appearance of components at the far end of the tube. It plots peaks on a graph, called a chromatogram. The peak areas are related to the concentration of the components. The time taken for a component to pass through the tube is called the retention time. This depends on the gas that is used how rapidly it flows as well as the temperature of the oven.

To calculate the relative amounts of a component in the sample the following formula is used:

Area =

Separated components can be passed into a mass spectrometer (Gas Chromatography–Mass spectrometry/ GC-MS) which record their mass spectra. Components may then be identified from their mass values and fragmentation patterns, or by using a computer to compare their spectra with those in a library.

GC-MS is used for security and in science and science, e.g. testing athletes for banned substances.

The GC detector will plot the results to produce a . A typical chromatogram is shown below:



Remember:

Type	Stationary phase	Mobile phase	Method of separation