Analysing Chromatograms – Paper Chromatography

Chromatography is a physical method that is good for **separating** & **identifying** things.

A **mixture** is not a pure substance, it is made up of two or more substances that are not chemically combined. Mixtures are easily separated (unlike compounds).

Uses of Chromatography
- To distinguish pure substances from impure substances
- Separate different dyes in an ink or food colouring
- Separate and identify amino acids

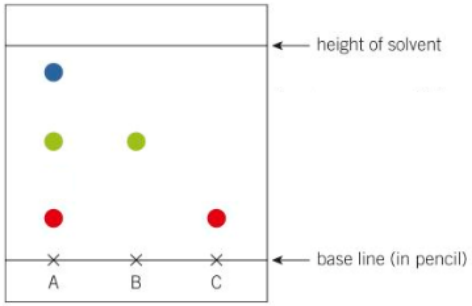
Chromatography always involves two phases, a **mobile** phase and a **stationary** phase.

In **paper chromatography,** the mobile phase is the **solvent**, the stationary phase is the **paper**.

A substance which has **stronger forces of attraction** to the mobile phase will move further up the paper.

A substance which has stronger forces of attraction to the stationary phase will not move very far up the paper in the same time.

If the unknown sample contains a mixture, then there will be more than one spot formed on the paper chromatogram.



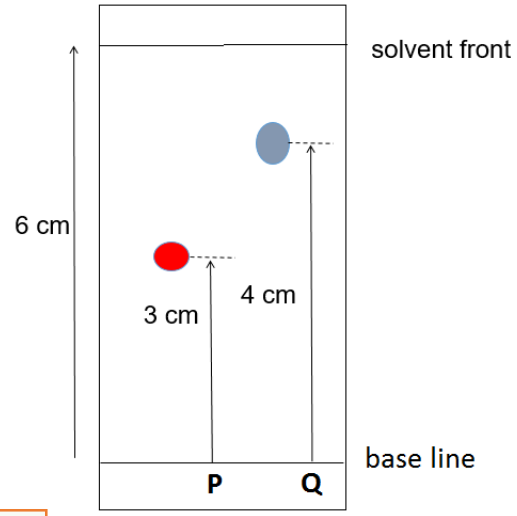
This chromatogram shows that there are 3 substances in mixture A. The mixture contains substances B, C and another unknown substance.

It is difficult for a scientist to predict the compounds present in a mixture. Hence knowing which pure compounds to compare a chromatogram with can be tricky. Hence it is more effective to measure data quantitatively using R_f (retention factor) values.

The blue unknown substance in the chromatogram above has the strongest attraction to the mobile phase as it moved the furthest up the paper.

There is a **dynamic equilibrium** between the mobile and stationary phases as the components constantly move between the two phases.

How quickly a substance moves up the paper depends on how it's distributed between the two phases. If it's more strongly attracted (more soluble) to the mobile phase then it **will dissolve better** and move up the paper faster.



For substance **P** $R_f = \frac{3}{6} = 0.5$

For substance **Q** $R_f = \frac{4}{6} = 0.67$

The further up the stationary phase that the spot travels, the larger the R_f value.

If separation is poor and the colourings have hardly moved from the baseline, then you should change the solvent.

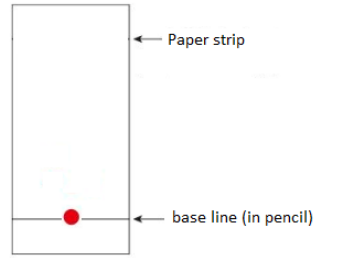
Examples of a polar solvent: Water or methanol
Examples of a non-polar solvent: Hexane.

Apparatus required to run a paper chromatogram:
Capillary tube, pencil, pipette, water, boiling tube, strip of chromatography paper.

A **pure** substance would only have **one spot** on a paper chromatogram.

The solvent used is usually water.
The stationary phase is paper.

Paper Chromatography **method**:
- Using a pencil, draw a base line near bottom of chromatography paper strip
- Add a spot of ink onto the line
- Place the strip of paper into a boiling tube/beaker of solvent (usually water), making sure ink spot doesn't touch the solvent.
- Place a lid on top of the boiling tube/beaker.
- Solvent moves up the paper, carrying the ink with it. When solvent has nearly reached the top, remove the paper strip.
- Using a pencil mark the solvent front.
- Allow the paper to dry.
- Calculate the R_f values



The point that the solvent has reached as it moves up the paper is called the **solvent front**.

A mobile phase is where the molecules can move. Usually a liquid. The stationary phase is where the molecules can't move (usually a solid or a really thick liquid.) The solvent (mobile phase) is drawn up the stationary phase (paper) using capillary action.

R_f values are stored in a library/database. As long as the **temperature** and **solvent** are the **same**, an unknown substance's R_f value can be compared with values in a database enabling identification of the substance.

R_f values are a ratio and are always **<1** as the distance moved by the substance is less than the distance moved by the solvent.

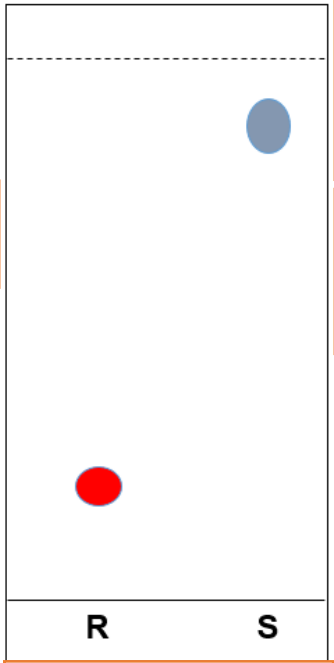
Retention factor (R_f) is calculated using the equation:

$R_f = \frac{\text{distance moved by substance}}{\text{distance moved by solvent (solvent front)}}$

The R_f value is **dependent on the solvent** used. If you change the solvent, then the R_f value will also change.

The difference in solubility in the mobile phase vs adsorption to the stationary is the reason why mixtures will separate out in a chromatogram.

There are quite a few different types of chromatography, paper chromatography is the only technique you need to know for GCSE.



R is less soluble in the mobile phase. S is more soluble (and hence has great attraction) in the mobile phase

Some organic compounds are **colourless** hence it is difficult to identify the spots on the chromatogram. Using a **UV lamp** or **spraying** the chromatogram with a **locating agent** will help identify the spots.

The **locating agent** that is sprayed onto a chromatogram to **identify amino acids** is called **ninhydrin**. Amino acid spots turn **blue-purple** when sprayed with ninhydrin.

Explaining chromatography **in an exam:**
- Paper chromatography involves two phases, a mobile phase and a stationary phase.
- The mobile phase is water, the stationary phase is filter paper.
- Separation depends on the balance between solubility In the mobile phase vs adsorption to the stationary phase.
- Compounds will move up the stationary phase at different speeds hence will have different R_f values.
- R_f value then compared to known R_f values in a database.