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| Chemistry experiment |
| Chromatography of dyes |



# Introduction

Chromatography is a technique used to separate non-volatile mixtures.

It works by exploiting different interactions between the dyes and the two components of the chromatography setup.

There are two main components in chromatography

The **stationary phase** – this is the (usually solid) base that does not move and the liquids pass through it.

The **mobile phase** – this is the fluid, containing the substances to be separated, that moves through the stationary phase

There are many different forms of chromatography.

For this analysis, we are using some simpler techniques: either paper chromatography or thin-layer chromatography.

## File:Chromatography tank.pngPaper chromatography

A coloured chemical sample is placed on a filter paper.

One end of the paper is placed in a solvent.

The solvent diffuses up the paper, dissolving the various molecules in the sample according to the polarities of the molecules and the solvent.

If the sample contains more than one colour, that means it must have more than one kind of molecule.

Because of the different chemical structures of each kind of molecule, the chances are very high that each molecule will have at least a slightly different polarity, giving each molecule a different solubility in the solvent.

The unequal solubilities cause the various colour molecules to leave solution at different places as the solvent continues to move up the paper.

The more soluble a molecule is, the higher it will migrate up the paper. If a chemical is very nonpolar it will not dissolve at all in a very polar solvent. This is the same for a very polar chemical and a very nonpolar solvent.

## Thin-layer chromatography

The process is similar to paper chromatography with the advantage of faster runs, better separations, and the choice between different stationary phases. It is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide or cellulose.

It is, however, significantly more expensive than paper chromatography and the theory is exactly the same.

## Rf values

The retardation factor (Rf factor) is the ratio of the distance travelled by the centre of a spot to the distance travelled by the solvent front

Calculate the Rf value for each pigment using the formula:

Rf = distance run by pigment (distance from pencil dot to top end of pigment)
       distance run by solvent (should be distance between the two pencil lines)

## Equipment and materials

**Each group will need:**

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| 1 sheet of chromatography paper | a small glass bottle (universal) |
| a rubber stopper for bottle with a slit about 3 mm deep along its narrow end\* | Dye sample(s) (in this case – pens) |
| pencil | Ruler & scissors |
| Chromatography solvent (ethanol) | Capillary tube(s) – for liquid dyes. |

*\* This is not essential but we have found it the easiest way to hold the paper while keeping the container sealed.*

*Having a lid on the container is essential, especially if you are using more volatile solvents like ethanol or propanone. The reason is that if there is no lid on there will be significant evaporation of solvent from the paper. Near the top in particular, which will interfere with the movement of the dyes. Having a lid means the atmosphere becomes saturated with solvent vapour which prevents this problem.*

**Instructions**

1. Take the sheet of chromatography paper. Cut the paper so that if fits into the tube without touching the sides – and is short enough that, when fitted into the slit in the bung will just about touch the bottom.
2. Using a ruler, draw a light pencil line across the paper about 1 – 2 cm from the bottom of the sheet.
3. Apply a spot of colour from one pen on the line. (Try to make sure it is fairly small - the colour spot should not exceed about 0.4 cm in diameter.)

*If you are using a liquid dye*

*Using a capillary tube, apply a small spot of the unknown dye mixture on the centre of the pencil line.*

1. If the spot seems to be too small or too light in colour, you can make it darker by applying a second spot of colour (or more) directly on top of the dry first one.

(It is necessary to allow the spot to dry between applications of colour in order to keep it small in size.)

1. Add the chromatography solvent to the glass universal, to a depth of about 0.5 cm.
2. Put the top of the paper strip into the slit in the bung (if you have not already) and insert it into the universal – the bottom of the paper should reach the solvent.

*(or use any other method of your choice to keep the paper upright in the container)*

1. Allow the solvent to move up the paper to within a few cm of the top.
2. Remove the paper from the universal and, using a pencil, mark the solvent front (the furthest level reached by the solvent). Measure the distance of the solvent from the pencil line.
3. You may need to leave the paper to dry, depending on the solvent. If so, place it on a paper on a paper towel to dry.
4. Measure and record the average distance from the front end of each dye colour to the pencil line where it started.

**learners then record their results and calculate Rf values**

## Hints and Extensions

**Pens** – in general dark coloured pens work better than light ones. Though green is often a mixture of yellow and blue.

**Solvent** – if you are using water-based pens then you can use water as your solvent. Though a salt solution (around 0.5%) works a little better.

If they are water resistant, you can experiment but ethanol (IMS) or a mixture of ethanol and water works quite well.

**Sweets** – you **can** try this with the colours from some sweets but in general the colour are paler and less easy to see. Green and purple are generally the best. To get the best colour, pur some skittles, M&Ms etc in a test tube and add a very small amount of distilled water. Shake the test tube: this will splash the water over the sweets for it to dissolve the colour and trickle down to the bottom.

## Safety

If you are using water-based pens or other sources of colour, there is no significant hazard.

If you are using a flammable solvent like ethanol or propanone, then there is a risk of fire so carry this out away from any sources of ignition.