



CHROMATOGRAPHY

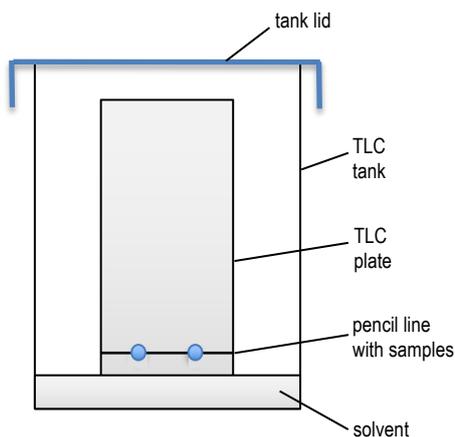
- Chromatography is one of the most commonly used techniques to separate and analyse compounds.
- There are many different forms of chromatography.
- All forms of chromatography consist of a
 - stationary phase that does not move
 - mobile phase that moves
- The substances in a mixture move at different speeds due to their different relative attraction to the mobile and stationary phases.

Type	Stationary phase	Mobile phase
column	powder (SiO_2 or Al_2O_3)	solvent
paper	absorbent paper	solvent
TLC	powder (SiO_2 or Al_2O_3) on a plastic sheet	solvent
gas (or gas-liquid)	powder packed in tube or liquid coated on tube-lining	inert gas (e.g. He, Ar, N_2)

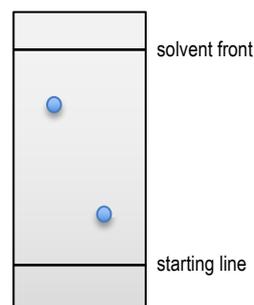
TLC (thin layer chromatography)

- TLC plates are plastic (or glass) sheets coated in SiO_2 (or Al_2O_3) powder.
- The samples are placed onto the powder as small dots on a pencil line.
- The plate is stood in a solvent (sometimes called eluent) in a tank / jar. The samples should be above the height of the solvent.
- The tank / jar should have a lid
 - to prevent solvent evaporating from the tank / jar
 - to maintain a constant atmosphere that is saturated with solvent vapour
 - to prevent solvent evaporating off the surface of the plate as it rises up
- The plate should not be moved during the experiment.
- The solvent soaks up the plate – the plate is removed once the solvent nears the top and the level that the solvent reaches marked with a pencil.

SET-UP



AFTERWARDS



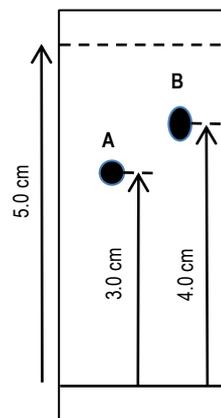
- The spots on the plate are usually colourless and cannot be seen with the naked eye.
- There are two common ways to make the spots visible:
 - view the plate under a UV light and mark the positions of the spots with a pencil (for all samples)
 - stain the spots with ninhydrin (specifically for amino acids)

R_f values

- We measure the relative distance that the solvent and sample being tested travel – this is the R_f value.

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

- Note that
 - we always measure the distance travelled from the start line (so that we are measuring the distances travelled by solvent and sample in the same time period).
 - we measure to the centre of the spots
- Substances that move furthest have the highest R_f values.
- How fast/far each compound moves depends on the relative attraction (affinity) of each compound to the mobile and the stationary phases.
- Molecules are more attracted to “like” molecules in terms of polarity. Polar molecules are more attracted to polar molecules. Non-polar molecules are more attracted to non-polar molecules.

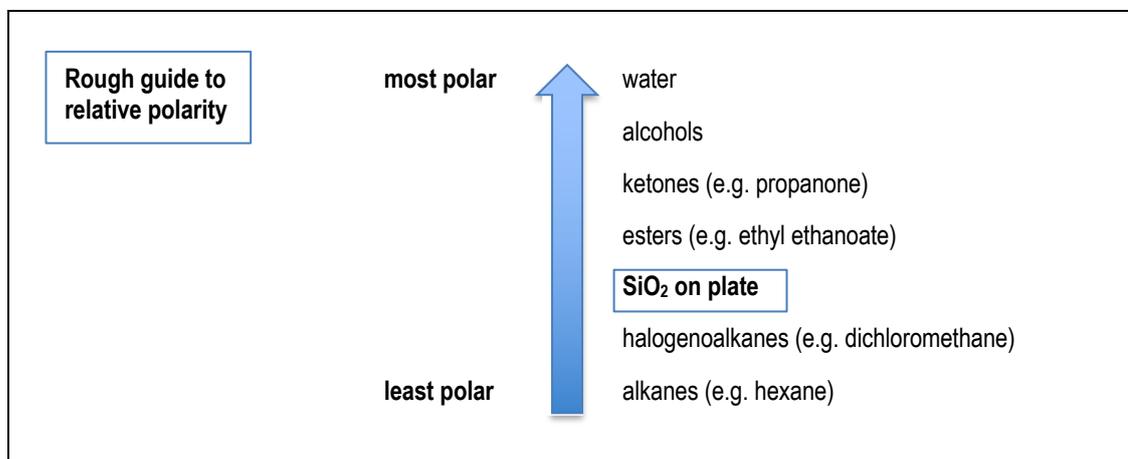


for substance **A**

$$R_f = \frac{3.0}{5.0} = 0.60$$

for substance **B**

$$R_f = \frac{4.0}{5.0} = 0.80$$



- How fast/far each compound moves depends on the relative attraction (affinity) of each compound to the mobile and the stationary phases. This is a rough guide and there are many deviations from this to very specific interactions between compounds, the solvent(s) and the silica.

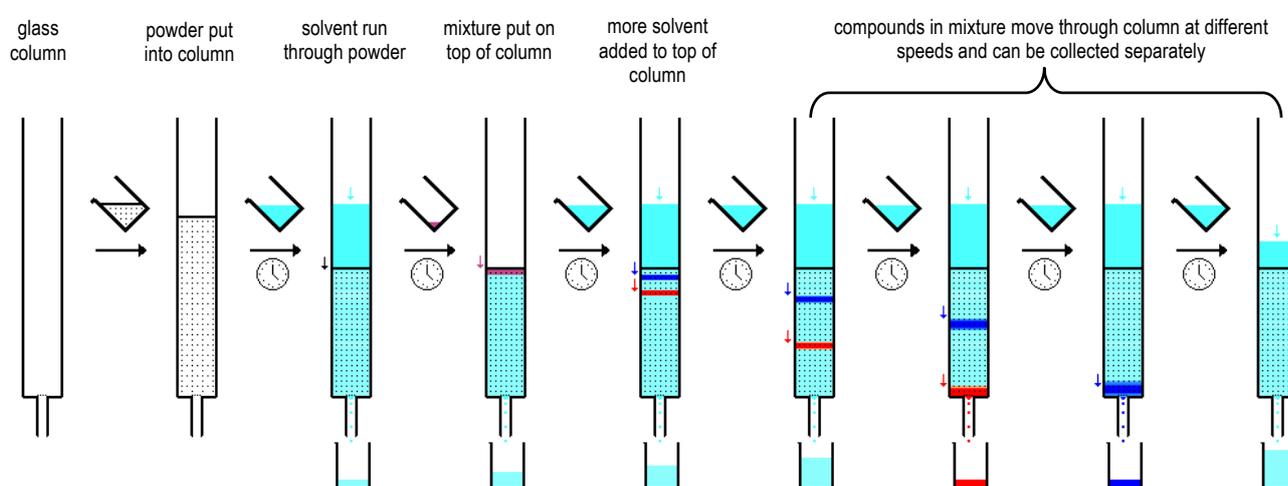
	Solvent is more polar than SiO ₂	Solvent is less polar than SiO ₂
Which substances move fastest (greatest R _f values)	Polar molecules (more attracted to the solvent)	Non-polar molecules (more attracted to the solvent)
Which substances move slower (lowest R _f values)	Non-polar molecules (more attracted to the SiO ₂)	Non-polar molecules (more attracted to the SiO ₂)

Paper chromatography

- This is rarely used above GCSE level. It is a simple and cheap version of TLC where the silica plate is replaced by absorbent paper.

Column chromatography

- This is a large-scale version of TLC that is used to physically separate compounds in a mixture rather than simply analyse what is in a mixture.
- The stationary phase is SiO_2 (or Al_2O_3) powder packed into a glass column.



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Gas / Gas-Liquid chromatography

- Gas (or gas-liquid) chromatography is commonly used to analyse gas, liquid or solid samples.
- Samples are heated and vaporised before they enter a long (coiled) column/tube.
- This column contains the stationary phase (many different substances are used, ranging from tubes packed with solid powders to tubes with an internal surface coating of a liquid)
- Samples are taken through the column using an inert carrier gas (e.g. He, Ar, N_2)
- Compounds travel through the tube at different rates and so leave the tube at different times. The time it takes a compound to travel through the tube is called the **retention time**.
- It is common for a mass spectrometer to be connected to analyse each substance as it leaves the column.

PROBLEMS

- 1) A mixture of pentanal and pentan-1-ol were separated by column chromatography using a silica powder with cyclohexane as eluent. Which of pentanal or pentan-1-ol is likely to reach the bottom of the column first? Explain your answer.

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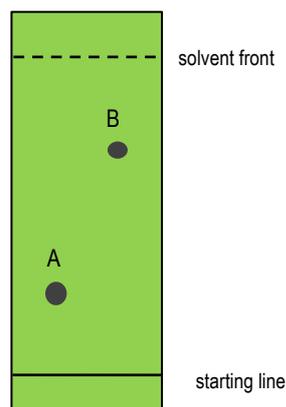
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- 2) The following TLC chromatogram was produced under a UV lamp.

- a) Calculate the R_f values for substances **A** and **B**.



- b) The plate was coated in silica powder and the solvent used was hexane. One substance was phenylamine and the other substance was tetrachloromethane. Which substance is likely to be which? Explain your answer.

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- 3) A mixture of opiates found in a sample of urine was analysed by gas chromatography.

- a) Which opiate had the longest retention time?

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- b) Explain why this substance had the longest retention time.

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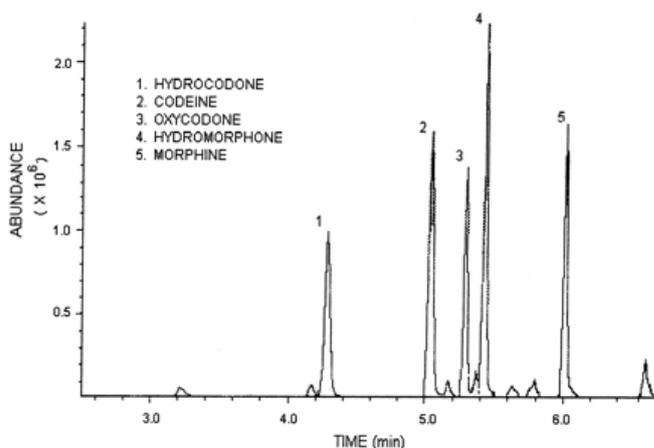
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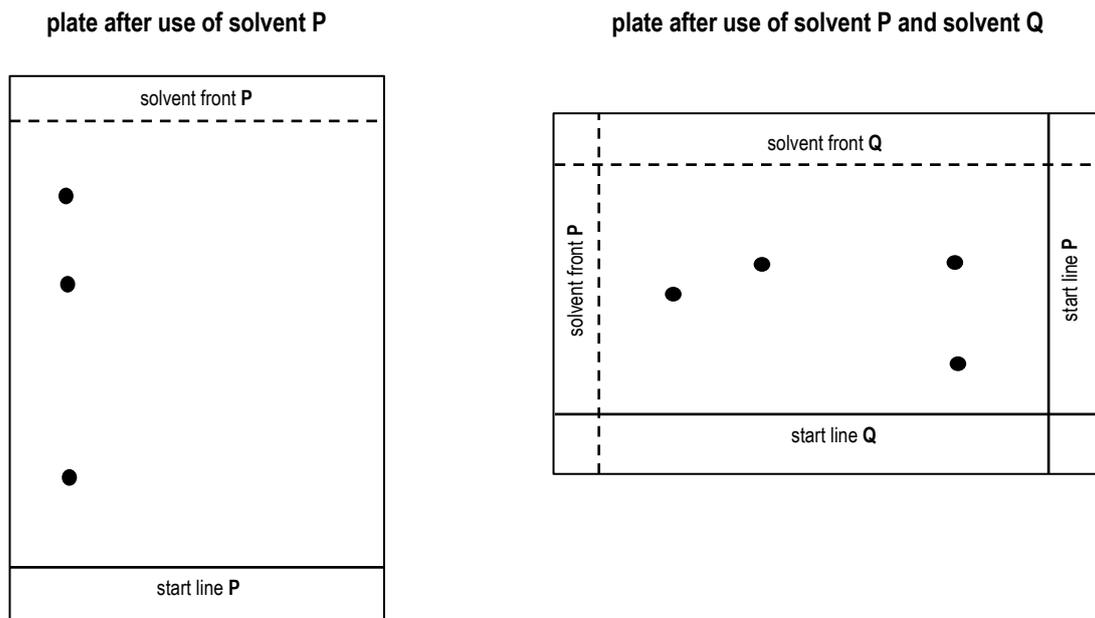
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- 4) A mixture of amino acids was analysed by TLC in solvent **P**. The plate is then turned anticlockwise and analysed using solvent **Q**. The results are shown.



a) How many amino acids are in the mixture?

b) Why were two solvents required to fully separate the amino acids?

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- 5) Tripeptides contain 3 amino acids joined together. The TLC of a tripeptide was done and the result shown.

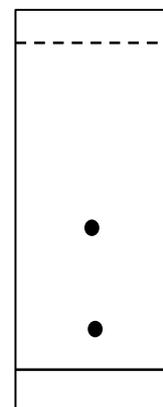
Suggest two possible reasons why there are two spots and not three in the chromatogram.

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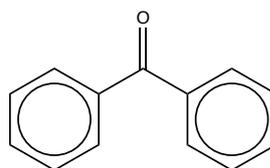
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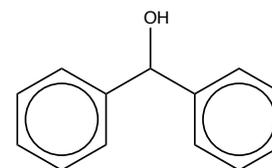
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- 6) The structure of two compounds are shown. Suggest which compound has the greatest R_f value in TLC using silica plates and methanol as solvent. Explain your answer.



benzophenone



benzhydrol

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