FACTFILE:
GCE CHEMISTRY

5.4 CHROMATOGRAPHY

Rewarding Learning

Chromatography

Learning Outcomes

5.4.1 describe and explain how paper (one-way and two way), thin-layer (TLC) and gas-liquid chromatography (GLC) is carried out qualitatively and quantitatively;

5.4.2 interpret GLC data in terms of the percentage composition of a mixture;

5.4.3 interpret one-way and two-way paper and TLC chromatograms including calculations of R_f values;

Chromatography

Chromatography is used to separate mixtures of soluble substances into their components. All types of chromatography have a **stationary phase** (a solid, or a liquid supported on a solid) and a **mobile phase** (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it

Methods of chromatography

1.Paper chromatography (one way and two way)
The paper can be thought of as cellulose fibers with a layer of water bonded to the surface. The stationary phase is the water that is adsorbed on the surface of the paper. The mobile phase is a solvent eg non-polar solvent such as hexane .

- Draw a base line using a pencil to close to the bottom of the paper
- Spot the samples onto the paper using a capillary tube. Allow the spots to dry. Repeat to make the spots concentrated.
- Place the paper in the tank containing a shallow amount of solvent, and allow the solvent to run up over the spot until the solvent reaches the top of the paper. This is called running the solvent. The tank is often sealed to saturate the atmosphere with solvent, this slows down the evaporation of solvent from the paper and prevents solvent loss.
- Run in solvent until the solvent front nears the top of the paper . The paper is taken out of the tank and the solvent front marked.
- If the substances to be separated are colourless, the spots are made visible by spraying with a locating agent, which reacts with them to make a coloured product. For analysis of amino acids in proteins, the chromatogram is developed by spraying ninhydrin in a fume cupboard and drying. Alternatively organic molecules absorb UV light so under UV light so the spots will fluoresce under UV light and be easily marked with a pencil.
- The distance travelled by the solvent front is measured. Then for each solute the **retardation factor,** R_f is calculated. It measures the distance traveled by the spot of solute relative to the distance moved by the solvent. Measure to the centre of the spot.

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

Notice that the R_f value must be less than one, as the spot cannot move further than the solvent.

- Compare R_f values. The R_f values can be used to assist in identifying the components by comparison with tables of values

Two substances may have **the same R_f value in a particular solvent**. It is possible to identify them by two way chromatography – run a chromatogram as mentioned previously. The first chromatogram is dried, the paper turned 90° and a second chromatogram is run at right angles using a second solvent, dried and R_f values calculated for the second solvent.

Paper chromatography, separating coloured inks.

© Andrew Lambert Photography/Science Phot Library

2. Thin layer chromatography (tlc)

Thin layer chromatography is carried out in an identical fashion to paper chromatography.

The stationary phase is a thin layer of silica (SiO_2) or alumina (Al_2O_3) coated on a flat support such as glass or plastic which is called the plate.

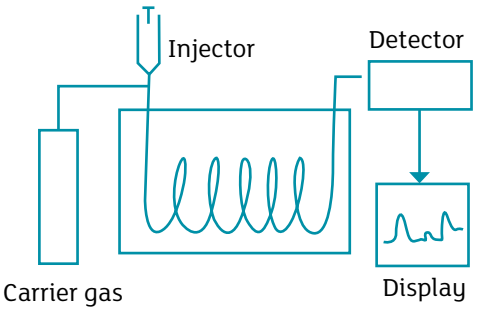
- Mark the base line on the thin-layer plate using a pencil
- Spot the samples onto the thin-layer.
- Run in solvent until the solvent front nears the top of the thin-layer plate.
- Ensure that the solvent starts below the pencil line.
- Use locating agents (if required).
- Compare R_f values.

3. Gas-liquid chromatography

Gas-liquid chromatography is used to separate volatile components in a mixture, such as organic compounds with low boiling points.

The stationary phase is a thin layer of solid (e.g. silicone polymer) or liquid (long chain alkane) coated on the inside of some capillary tubing which acts as an inert solid support. The tubing is the chromatography column and it is wound in a coil that fits inside a thermostatically controlled oven. The mobile phase is an unreactive gas such as helium or nitrogen – it is called the carrier gas.

The more volatile a sample, the faster it passes through the machine.



- Inject the sample into the apparatus, it is heated and vaporised and carried along the stationary phase by the carrier gas.
- A detector records each component as it leaves the column and traces out a graph, the area under each peak being proportional to the amount of that component. **The time taken from injection until a component reaches the detector is called the retention time.**
- Compare retention times to help identify an unknown sample.
- Some components interact less with the stationary phase than others and emerge from the column ahead of the others i.e different components separate as they **have different retention times**.

Interpreting the output from the detector

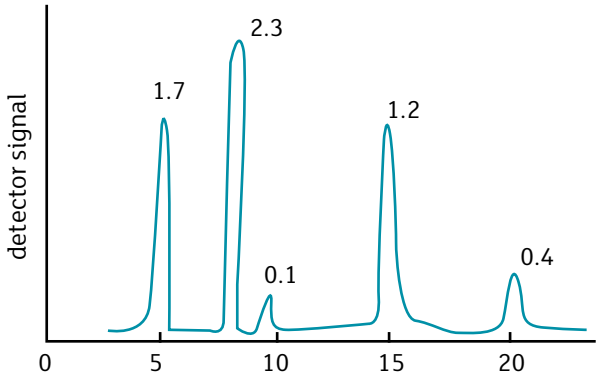
The output will be recorded as a series of peaks - each one representing a compound in the mixture passing through the detector. The retention times can help to identify the compounds present – by comparison with known values.

The areas under the peaks are proportional to the amount of each compound which has passed the detector, and these areas can be calculated automatically by the computer linked to the display and are used to calculate percentage

composition of the mixture, which can be useful when monitoring an equilibrium reaction. If a sample of a substance is 80% pure, then there should one peak with 80% of the area within it and other peak(s) with 20% of the rest of the area.

Example

The numbers above the peaks indicate the relative area under the peak which allows us to work out percentage composition.



Total of all areas = 1.7 + 2.3 + 0.1 + 1.2 + 0.4= 5.7

Peak at 5 minutes

percentage composition = $\frac{1.7}{5.7} \times 100 = 29.8\%$

Peak at 8 minutes

percentage composition = $\frac{2.3}{5.7} \times 100 = 40.4\%$

Peak at 10 minutes

percentage composition = $\frac{0.1}{5.7} \times 100 = 1.8\%$

Peak at 15 minutes

percentage composition = $\frac{1.2}{5.7} \times 100 = 21.1\%$

Peak at 20 minutes

percentage composition = $\frac{0.4}{5.7} \times 100 = 7.0\%$

Revision Questions

1 A mixture of amino acids may be separated using paper chromatography.

(i) Explain the term R_f value as applied to paper chromatography.

.....[1]

(ii) Explain what a low R_f value indicates about a particular amino acid.

.....

.....

.....[1]

2 Succinic acid can be analysed by converting it to the diethyl ester and submitting the ester to GLC analysis.

(i) Why is it better to use the ester rather than the acid in GLC analys

.....[1]

(ii) Explain the results expected if the sample of the ester was 90% pure.

.....

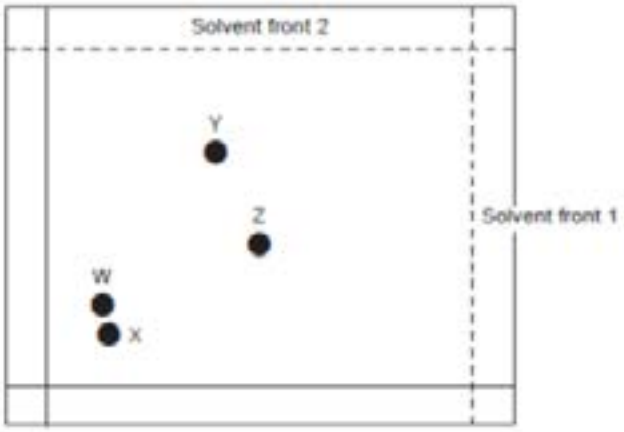
.....[1]

3 Which one of the following is **not** true for gas-liquid chromatography of a mixture?

A The mobile phase is a liquid and the stationary phase is a gas
B The molecules in the mixture have characteristic retention time
C More volatile components of a mixture have a low retention time
D The percentage composition of the mixture can be determined [1]

Revision Questions

4 The chromatogram below was produced by two-way paper chromatography of a mixture of amino acids.



The table below gives the R_f values of some amino acids

Amino acid	R_f values	
	Solvent 1	Solvent 2
Alanine	0.51	0.38
Asparagine	0.63	0.21
Isoleucine	0.44	0.72
Glycine	0.12	0.26
Lysine	0.18	0.14

Which one of the spots, W, X, Y or Z is glycine?

A W
B X
C Y
D Z

[1]

© CCEA 2017