ANALYTICAL CHEMISTRY

Chromatography

LO : understand qualitatively paper, high performance liquid, thin layer and gas/liquid chromatography in terms of adsorption and/or partition and be able to interpret data from these techniques.

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Chromatography

- What is Chromatograpy?
- http://www.youtube.com/watch?v=SdYb6GgBQ7s

- Four techniques will be described here:
  - paper chromatography (PC),
  - thin layer chromatography (TLC),
  - gas/liquid chromatography (GLC) and
  - high performance liquid chromatography (HPLC).

The basic principles and techniques

- All chromatographic methods use the same **principle of a mobile phase** (a liquid or a gas) **moving past a stationary phase**.

- **Stationary phase** may be a **solid** onto which the **solutes are adsorbed (TLC, and some GLC and HPLC)** or a **liquid which is held in a thin film of the surface of an inert solid (PC, GLC and HPLC).**

The basic principles and techniques

- The **solute molecules partition themselves between the stationary phase and the moving phase**.

- Unlike solvent extraction, however, the **partition is not a true equilibrium**, since the **mobile phase is constantly moving past the area on the stationary phase where a particular solute is adsorbed/dissolved**.

- The **mobile phase should never become saturated with the solute**.
- http://www.youtube.com/watch?v=SdYb6GgBQ7s

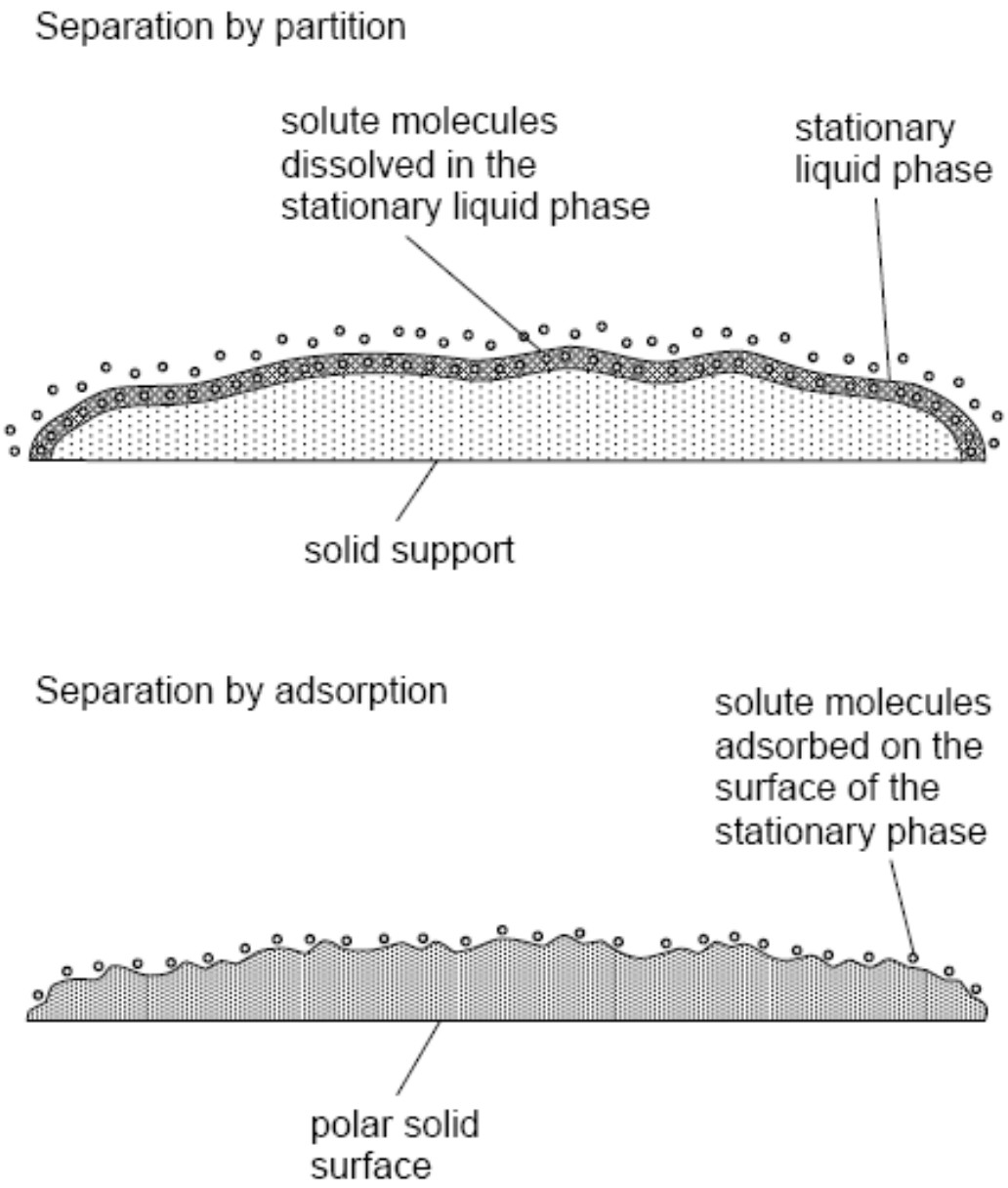


Figure 2.29 – the two types of chromatographic separation – partition and adsorbtion

Paper Chromatography

- The **mixture to be separated** and analysed is **dissolved in a solvent**, such as water or ethanol.
- a small spot of the solution is placed about 1 cm from the edge of a rectangular sheet of chromatography paper.
- spots of "reference" compounds may also be applied at the same distance from the edge.
- Solvent is allowed to evaporate.
- http://www.youtube.com/watch?v=gpFb635N2wo

Paper Chromatography

- The **solutes adsorbed into the fibres of the paper** (often **by means of hydrogen bonding to the OH groups of the cellulose fibres, or more likely to the H_2O molecules that are still associated with cellulose.**

- **Stationary phase – water molecules on cellulose.**

- The edge of the sheet - immersed in the chromatography **solvent** (the **mobile phase**).

- **Capillary action** draws the liquid up the sheet, and as it passes the point where the spot has been adsorbed, the **compounds in the mixture will partition themselves between the cellulose surface and the moving solvent**.

Paper Chromatography

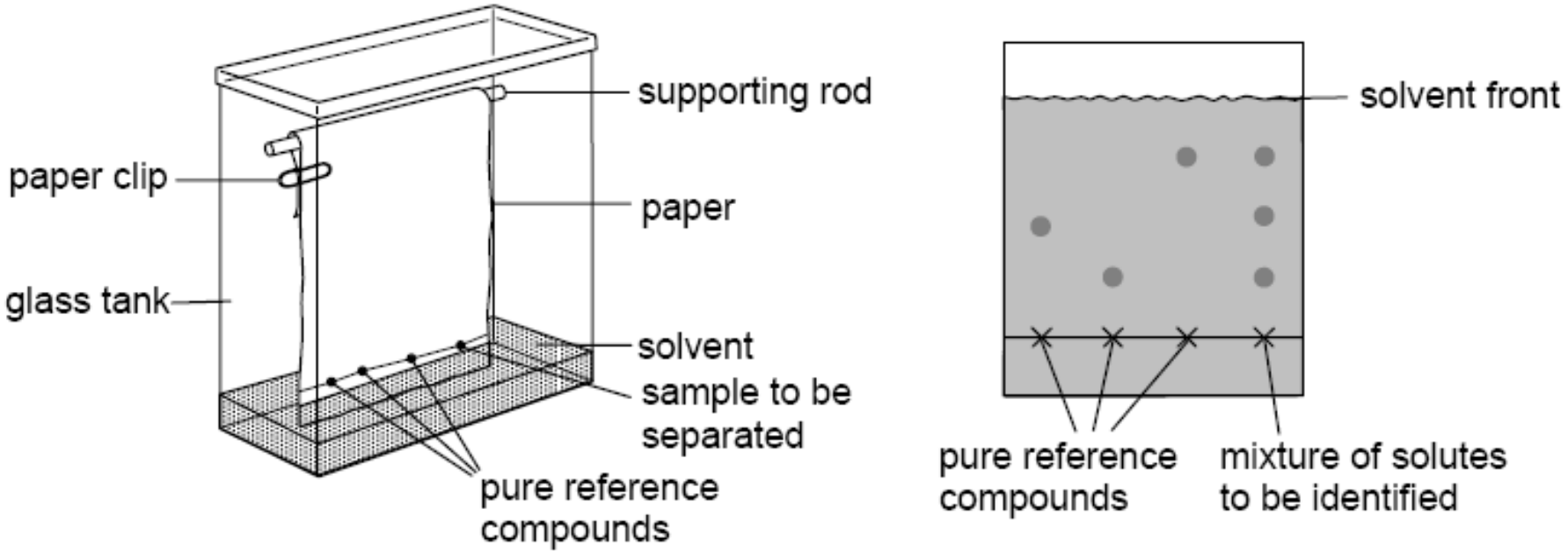


Figure 2.30 – paper chromatography and the resulting chromatogram

Paper Chromatography

- The **solvent is usually less polar than the cellulose surface** and its associated water layer;
  - so **polar, strongly hydrogen bonded compounds** only **travel to a small degree**
  - **less polar** and **less hydrogen bonded** compounds **travel a larger distance**.

Paper Chromatography

- Once the **solvent has reached the top end** of the sheet of paper, the **paper is removed** and the **solvent is allowed to evaporate**.

- **All solutes will now be re-adsorbed onto** the area of **the paper** where they had reached, and their presence can be **detected by their colour**, **or** by their **UV absorbance.**

- **For colourless compounds**, an easy way of visualising the spots is to place the paper in a beaker with a few **iodine crystals**.

Paper Chromatography

- **Iodine vapour** is preferentially **absorbed by the less polar solute spots**, **making them appear brown**.

- Paper can be sprayed with a dilute solution of a reagent that forms colours with the compounds contained in the spots.

- **Ninhydrin** is used to visualise **amino acids** and small peptides;
- **Molisch's reagent** can be **used for sugars in general**;
- **Tollens' reagent** can be used for **reducing sugars** such as **glucose** and **maltose**.

Paper Chromatography

- The ratio R_f , called the retention ratio (or retardation factor), is defined by

$$R_f = \frac{x}{y} = \frac{\text{distance moved by solute}}{\text{distance moved by solvent}}$$

- Each solute or component has a characteristic R_f value for a given solvent, hence it can be used to identify a possible component.

- http://www.youtube.com/watch?v=gpFb635N2wo

Two-way Paper Chromatography

- Sometimes, different compounds may have very similar R_f values in a particular solvent, and so no separation can be achieved.
- Using different solvent, the two compounds can be separated.
- One solvent is used first, and the solvent front allowed to reach the far edge of the paper, after which the paper is placed with the left side at the bottom for a second development using a different solvent.

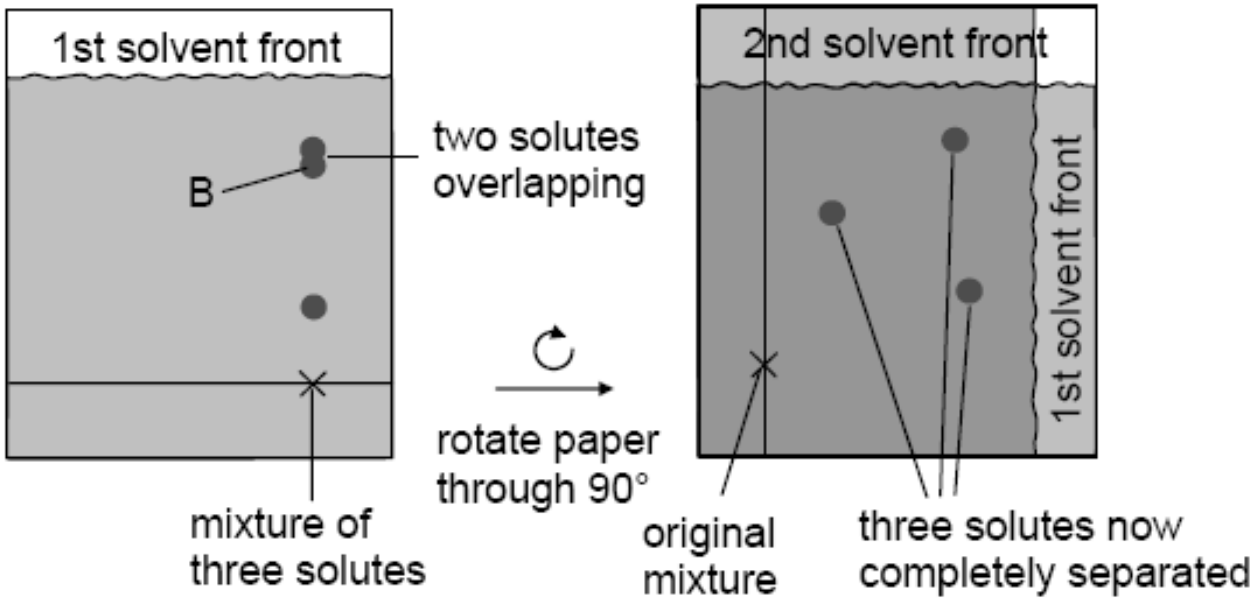
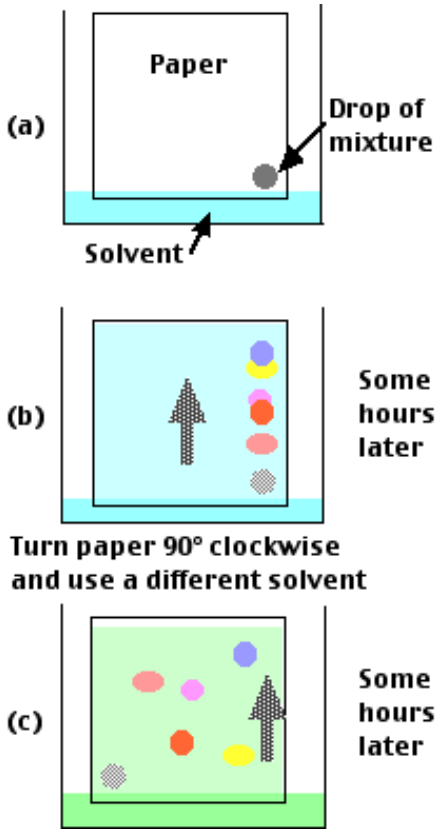


Figure 2.32 – two-way paper chromatography

Two-way Paper Chromatography

- The spots are then more widely dispersed and can be recognised from their positions on the final chromatogram.

- Used to identify the amino acids obtained from the hydrolysis of a protein



Thin Layer Chromatography

- Similar to paper chromatography
- **Stationary phase** is a **thin layer of a solid** such as **alumina or silica** supported on an inert base such as glass, aluminium foil or insoluble plastic.

- Silica or alumina is made into slurry with water and coated on the surface of glass plate.
- Mixture to be analysed is 'spotted' at the bottom of the TLC plate and allowed to dry.

- Plate is placed in a closed vessel containing solvent (the mobile phase) so that the liquid level is below the spot.

Thin Layer Chromatography

- Solvent ascends the plate by capillary action - liquid filling the spaces between the solid particles.

- The **partition** here is **between** the **solute adsorbed onto the SiO_2 or Al_2O_3 particles (via polar/hydrogen bonded interactions,** but also **some acid/base attractions**, since SiO_2 is slightly acidic, and Al_2O_3 slightly basic) and the solute dissolved in the moving phase.

- **More polar molecules travels more slowly** up the thin layer of alumina or silica as they are adsorbed more strongly onto the surface of stationary phase.

Thin Layer Chromatography

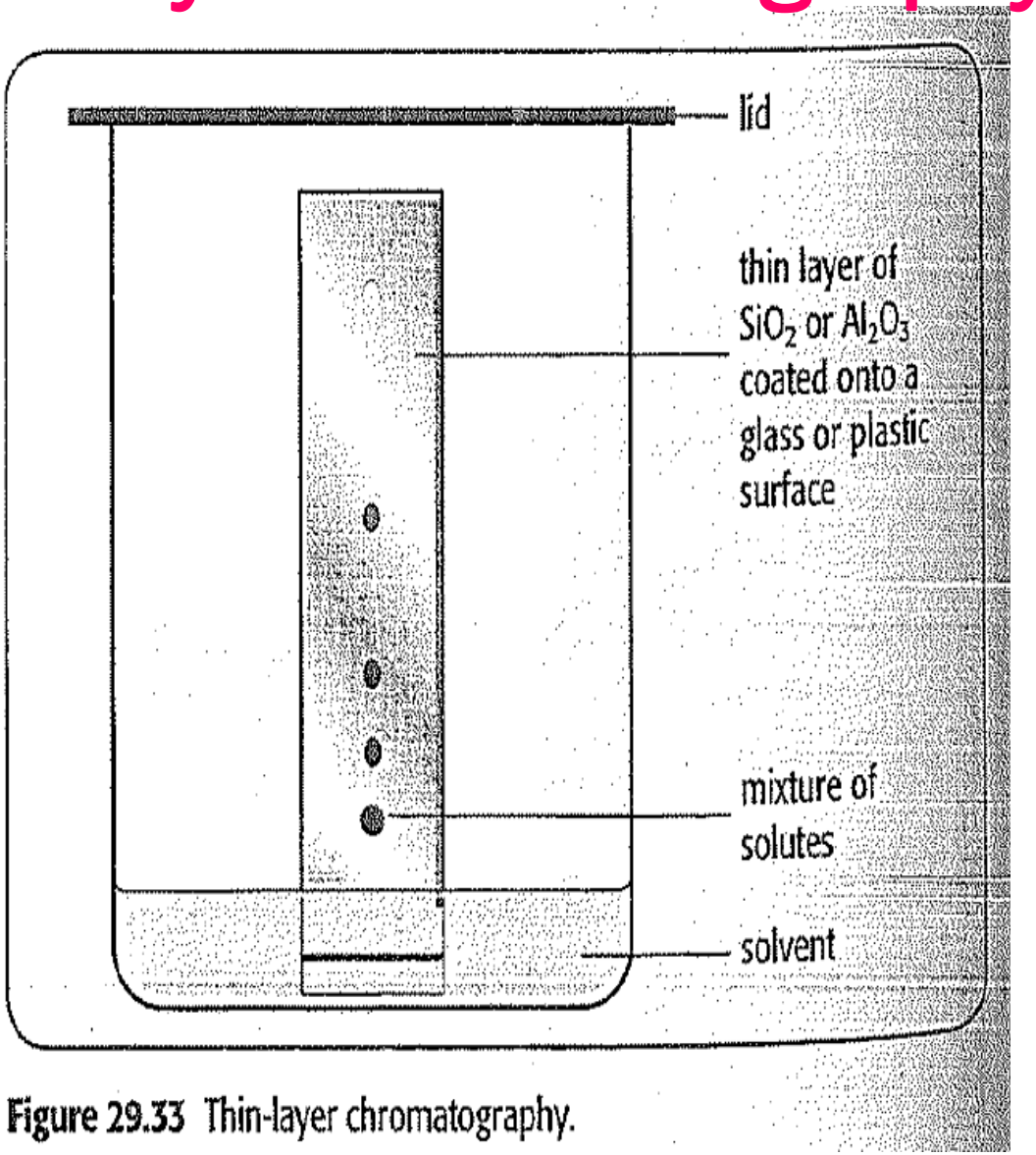


Figure 29.33 Thin-layer chromatography.

Thin Layer Chromatography

- Advantages :
- **results are more reproducible**,
- **separations are very efficient because of the much smaller particle size of the stationary phase.**
- can be used on **smaller samples** – useful in forensic science.
- **Faster** than paper chromatography.

- TLC has applications in industry in:
  - **determining the progress of a reaction by studying the components present**; and
  - in **separating reaction intermediates**.

Thin Layer Chromatography

- The **ways in which spots of colourless compounds** on a TLC plate can be visualised are **similar to those used for PC**

- **Silica** or **alumina** is **impregnated with a fluorescent insoluble compound that absorbs UV light and emits it as visible light**.

- When placed **under a UV lamp**, the plate emits a bright white light except where a UV absorbing compound is situated. Here, a **dark spot is observed**.

Gas/Liquid Chromatography

- **Gas** as the **mobile phase**.
- **Non-volatile liquid coated onto small inert particles** as the **stationary phase**.
- The **particles are packed into a narrow glass column** a **few mm in diameter** and between **1 m and 3 m long**.
- The **column is mounted inside an oven** whose temperature can be controlled, or even programmed to increase as the separation is underway.

Gas/Liquid Chromatography

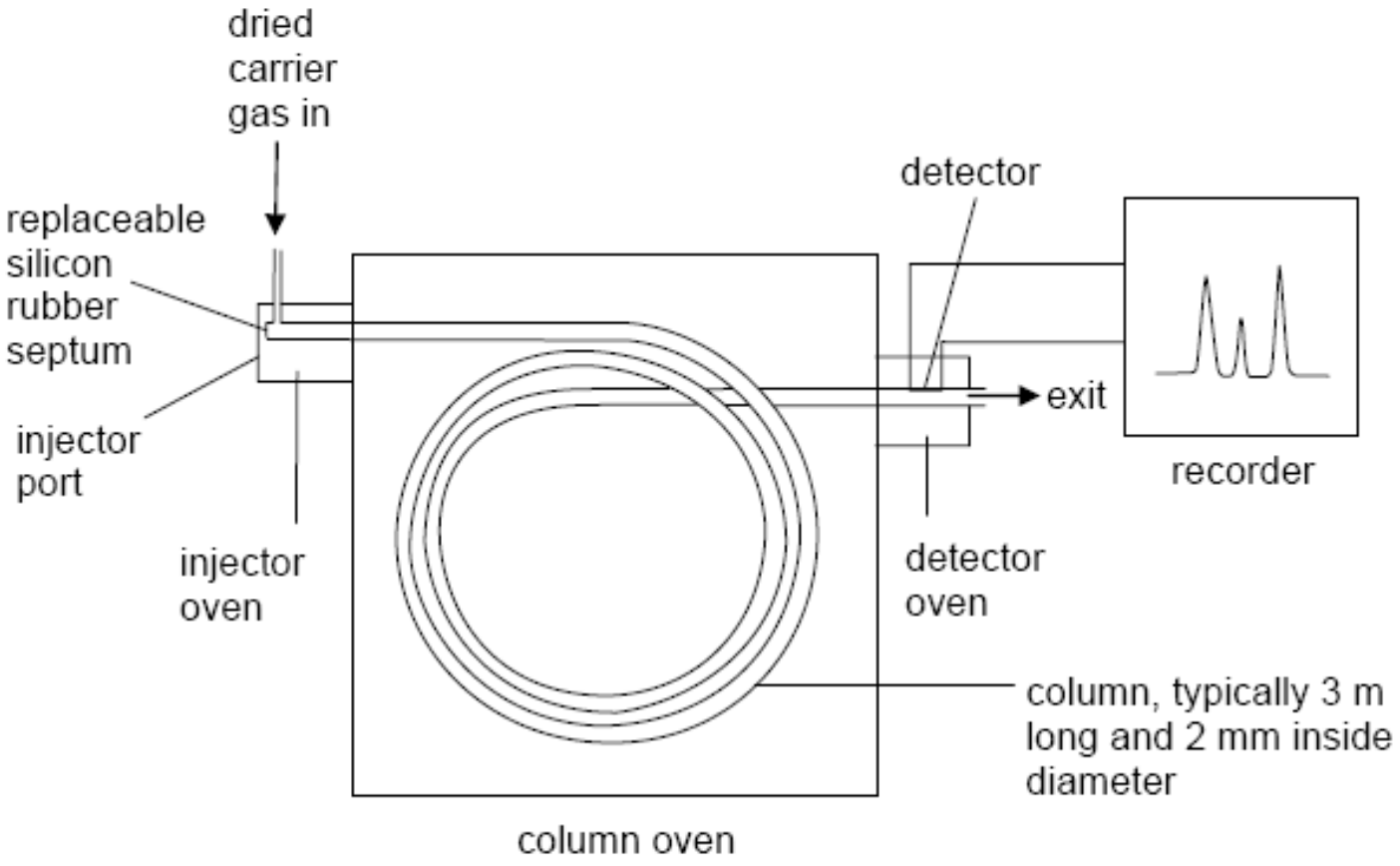


Figure 2.33 – GLC apparatus

Gas/Liquid Chromatography

- For separation or identification the **sample must be either a gas or have an appreciable vapour pressure at the temperature of the column**.

- The **sample is injected through a self-sealing disc** (a rubber septum) **into a small heated chamber** where it is **vaporised** if necessary.

- Although the **sample** must **all go into the column as a gas, once it is there the temperature can be below the boiling point of the components as long as they have appreciable vapour pressures inside the column**.

Gas/Liquid Chromatography

- This **ensures that all the fractions pass through the column over a reasonable time span**. The injector oven is usually 50-100 °C hotter than the start of the column.

- The **sample is then taken through the column by an inert gas** (known as the carrier gas) such as **helium or nitrogen**, which **must be dry** to avoid interference from water molecules.

- Dried by passing it through anhydrous copper(II) sulphate or self-indicating silica

Gas/Liquid Chromatography

- **Unwanted organic solvent vapours can be removed by passing the gas through activated charcoal.**

- **The column is coiled so that it will fit into the thermostatically controlled oven.**

Gas/Liquid Chromatography

- The **temperature of the oven is kept constant for a straightforward separation**,
- If there is a large number of components, or if they have similar affinities for the stationary phase relative to the mobile phase, then it is common for the temperature of the column to be increased gradually.
- Gives a better separation if the boiling points of the components are close.
- Faster separation if some components are relatively involatile

Gas/Liquid Chromatography

- The **fractions progress to the end of the column, and then to a detector**.

- Two types of detector:
  - thermal conductivity detectors and
  - **flame ionisation detectors (FID)**

- It is the one that is most commonly used, and is particularly useful for **detecting organic compounds**.

Gas/Liquid Chromatography

- Once a mixture has been separated by GLC its components need to be **identified**.

- Retention time is the **time it takes for the components to reach the detector once they have been injected into the column**.

Gas/Liquid Chromatography

- **retention times** and will vary depending on each of the following:

  - the **flow rate of the carrier gas**;
  - the **temperature** of the column;
  - the **length and diameter of the column**;
  - the **nature of and interactions between the component and the stationary and mobile phases;** and
  - the **volatility of the solute**.

Gas/Liquid Chromatography

- **Each substance to be identified by GLC is run through the column so that its retention time can be determined.**

- For compounds of completely unknown structure or composition the components must be collected individually and then analysed by using another method – e.g. mass spectrometry.

Gas/Liquid Chromatography

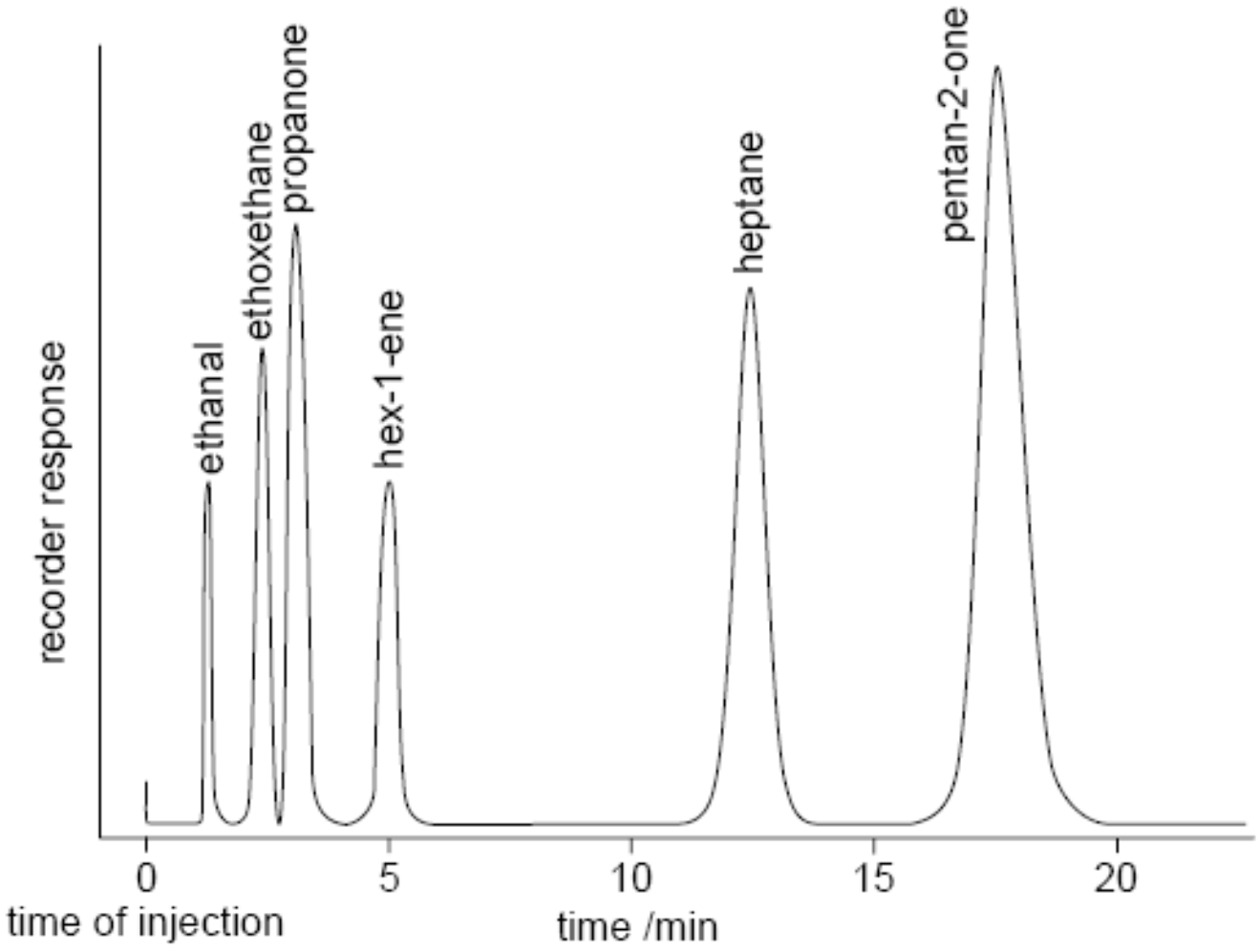


Figure 2.35 – a GLC chromatogram of a mixture of organic chemicals

High Performance Liquid Chromatography

- Very **similar to GC**.
- Main differences are that the **mobile phase is a high-purity solvent**.
- **Columns are shorter** (10 – 30 cm) and the **components are usually detected by measuring the absorbance of UV radiation through a microcell at the end of the column**

High Performance Liquid Chromatography

- The **stationary phase in an HPLC column normally consists of uniform porous silica particles** of diameter 1 x 10^{-6} m, with surface pores of 1 x 10^{-9} m diameter **to increase the surface area**.

- Smaller particles have better separation efficiency because the **solute can equilibrate more rapidly between the two phases**

- Sometimes the particles are **coated** with molecular fragments of a solvent having particular **polar groups**, joined to the silica by **covalent bonds** (ensures they do not dissolve in the mobile phase).

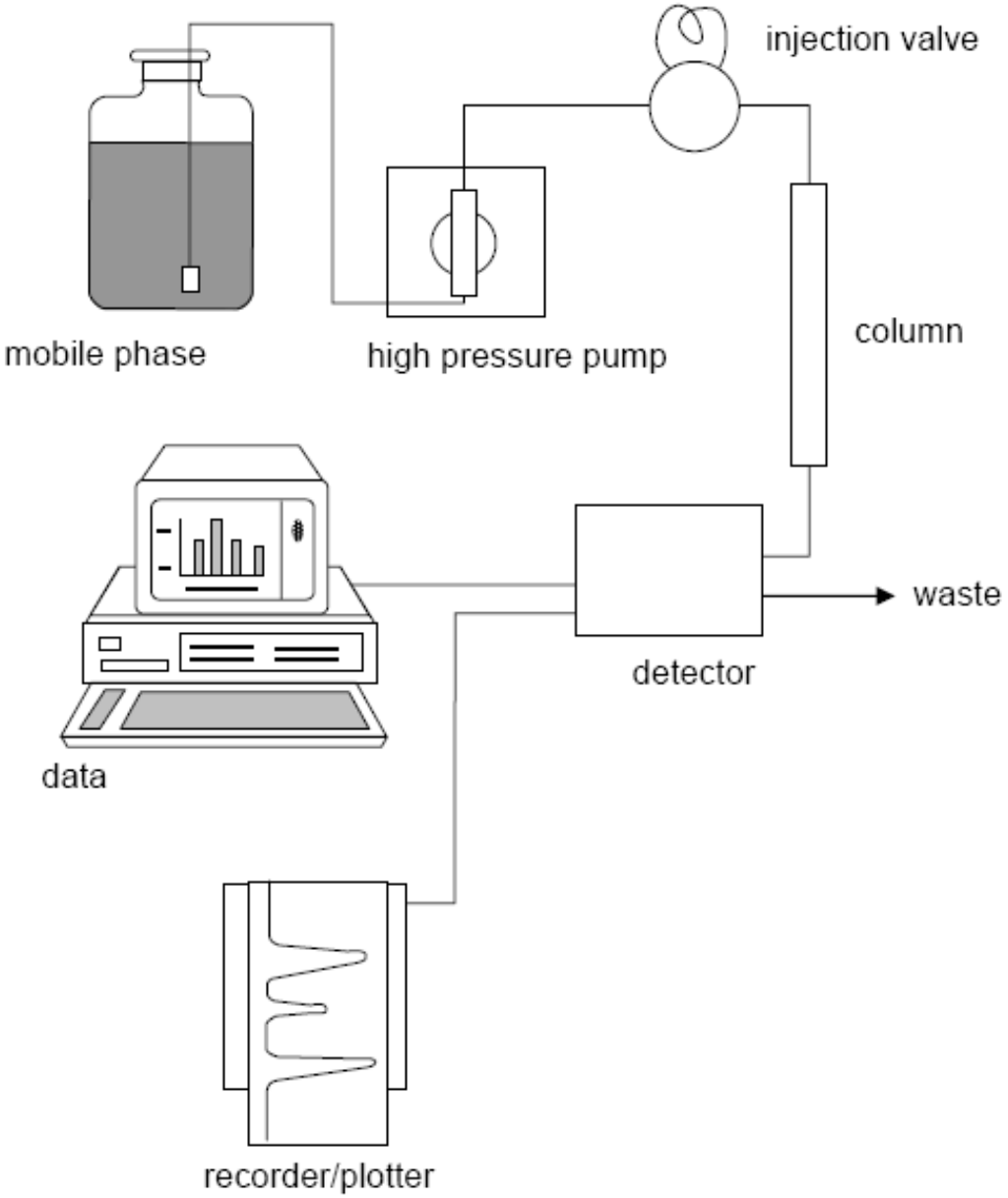


Figure 2.36 – HPLC apparatus

High Performance Liquid Chromatography

- To ensure reproducibility of retention times, various factors have to be controlled exactly.

- A **constant flow rate is maintained** by special twin cylinder reciprocating pumps, **generating steady precise pressures of up to 100 atmospheres**.

- Even with such high pressures, the **flow rate through the column is small** (about 2 cm^3 min^{-1}), **due to the tightly packed small particles** of the stationary phase.

High Performance Liquid Chromatography

- Nevertheless, because the column volume is small the **timing of the injection of the sample must be precise if an accurate retention time is to be measured**.

- Application :
- Medical research – separate peptides and proteins.
- Analyse urine samples from athletes for steroids or stimulants.
- Monitoring pollutants in atmosphere and river, eg. Pesticides.
- By food agencies to check accuracy of data on food labels.