Week 5 Chromatography & Spectroscopy

Chromatography

Used mainly to separate the substances present in an organic mixture, or to identify a substance.

Applications include the identification of:

- Drugs present in blood
- Sugars in fruit juice
- Hydrocarbons in oil
- Pollutant gases in exhaust fumes
- Pesticides in water and soil

Figure 6.1
A simple chromatogram. One end of this chalk was dipped in black ink before being placed in the beaker of water. The black ink separates into its different coloured components as it rises up the chalk.

How chromatography works

All methods of chromatography have:
- A stationary phase
- A mobile phase

In the chalk example, the stationary phase is the chalk and the mobile phase is the water.

As components of the ink are swept forward over the stationary phase, the continually adsorp to the stationary phase and desorb into the mobile phase.

The rate of movement of each ink component up the chalk depends on
- The strength of absorption onto the stationary phase
- How readily it dissolves in the mobile phase.

In Fig. 6.1 the blue dye has moved faster up the chalk than the red dye resulting in their separation.
This is because the blue dye is more soluble in the mobile phase, and bonds less strongly with the stationary phase than the red dye.

Thin-layer chromatography

Used for qualitative analysis

- A thin-layer of fine powder (*e.g.* aluminium oxide) is spread on a glass or plastic plate. This is the chromatography plate.
- A solution of the sample is spotted onto one end of the plate (the origin).
- One edge of the plate is submerged in a solvent with the sample spot above the solvent.

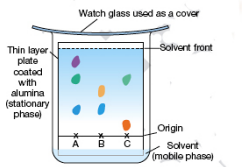
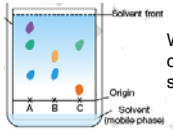


Figure 6.2
Thin-layer chromatography of three different food colours (A, B and C). A number of chemicals once used as food colours are now regarded as hazardous and can be detected by this method.

Interpreting chromatograms of TLC

The identity of chemicals in the mixture can be identified in 2 ways:

1. Method 1: Running standards of known chemicals on the same chromatogram as the unknown sample.
    a. With this method it is necessary to have an idea of the chemical that you are looking for in the sample.
    b. A pure sample(s) of the chemical being tested for needs to be run on the same chromatogram.
    c. If spots of unknowns moving the same distance as the pure samples are *likely* the same chemicals.



Which spots in the chromatogram on the left are likely to contain the same chemicals?

2. Method 2: Calculating the R_f value of the sample.

   a. Identification by distanced travelled by components along the stationary phase compared to the distance travelled by the solvent front.
   $R_f = \frac{\text{distance moved from origin by component}}{\text{distance moved from origin by solvent}}$
   b. R_f values will always be less than one
   c. The component most strongly absorbed onto the stationary phase moves the shortest distance and has the lowest R_f value.
   d. Each component has a characteristic R_f value under each chromatogram condition.
   e. By comparing R_f values of of unknowns with those of known substances under identical chromatographic conditions, the components in mixture can be identified.
   f. The distance moved by the solvent is not critical as the proportion of the distance moved remains the same.

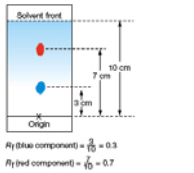


Figure 6.3
Calculation of the R_f values of components on a chromatogram.

key question

1 An extract from a plant was analysed using thin-layer chromatography with a non-polar solvent. The chromatogram obtained is shown in Figure 6.4.

Figure 6.4
Chromatogram for Question 1.

Table 6.2 gives the R_f values of some chemicals commonly found in plants.

TABLE 6.2 R_f values of some plant materials

Chemical	R_f
Xanthophyll	0.67
β-carotene	0.82
Chlorophyll *a*	0.48
Chlorophyll *b*	0.35
Leutin	0.39
Neoxanthin	0.27

a Measure and record the distance from the origin to the centre of each band, and the distance of the solvent front from the origin.
b Calculate the R_f value of each band.
c Compare R_f values for the bands to the R_f values in Table 6.2 and name the chemicals present in the extract.
d If water had been used as the solvent, would the chromatogram be likely to have a similar appearance? Explain.

Column chromatography

- Stationary phase: a solid, or solid thinly coated with a viscous liquid.
- A solvent is the mobile phase

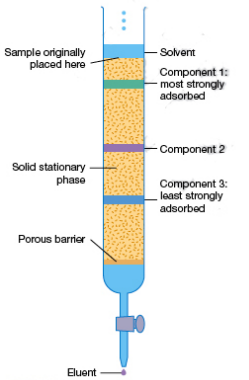


Figure 6.5
Using column chromatography to separate the components in a mixture.

High Performance Liquid Chromatography (HPLC)

- Makes possible extremely sensitive analysis if a wide range of compounds.
- Used routinely for pharmaceutical and industrial analyses.

Differs from traditional chromatography:

- Particle size often 10 - 20 smaller than column chromatography.
- This allows for more frequent absorption and desorption of the components, giving better separation of similar compounds.
- Small particle size = high flow resistance. Therefore, the solvent is pumped under high pressure up to 14,000 KPa.
- A range of solids are available for HPLC columns, some with chemicals bonded to their surfaces to improve the separation of particular classes of compounds.

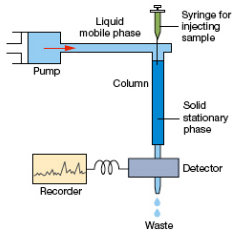
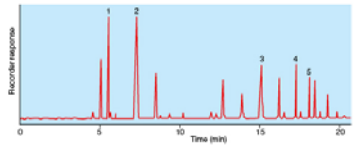


Figure 6.6
The construction of a high performance liquid chromatograph.

- The components are usually detected by passing the eluent through a beam of UV light in a detector.
- Many organic compounds absorb UV and so attenuate the signal in the detector.
- The signal is recorded on a paper chart moving at constant speed. This becomes the chromatogram.



- Time taken for a component to pass through the column is called the retention time, R_t
- R_t is characteristic of each component for the conditions used in the paricular chromatography. It analogous to R_f.
- R_t is used to identify the components associated with peaks in the chromatogram.
- The relative amounts of each component are determined by comparing areas under the peaks with areas under peaks of known standards.

Gas Chromatography (GC)

- Most sensitive of the chromatographic methods. Detects as little as 10^{-12} g of a compound.
- However, is limited to compounds that can be vaporised.
- Extreme sensitivity of GC makes it ideal for the analysis of trace contaminants in samples *e.g.* detection of illegal performance-enhancing drugs in the samples provided by athletes.
- 2 types of GC: Gas-Liquid (GLC) and Gas-Solid chromatography (GSC).

GC has the following features:

- The mobile phase is a gas, generally nitrogen, called carrier gas.
- The sample is introduced at the top of the column via an injection port which is heated to instantly vaporise the sample which is then swept into the column by the carrier gas.

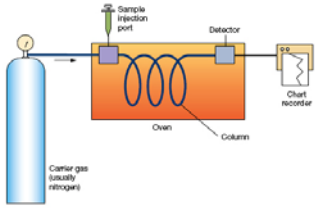


Figure 6.9
A gas chromatograph.

- The column is a loop that can be up to 2 – 3 m in length. In GLC it is packed with a porous solid coated with a liquid hydrocarbon or ester with a high boiling point. In GSC, the packing material is an adsorbant solid such as silica gel or alumina.
- The column is mounted in an oven and heated.

- The components repeatedly pass in and out of solution with the stationary phase. The least soluble are swept out first by the carrier gas.

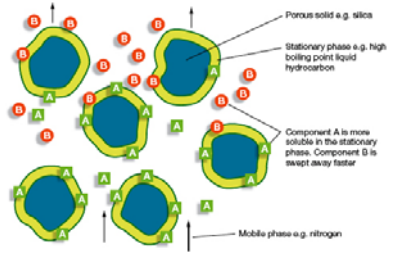
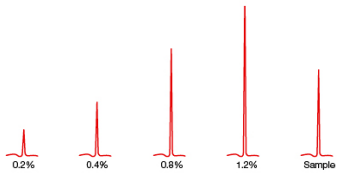


Figure 6.10
Section through a GLC column.

- The most useful detector is the Flame Ionisation Detector (FID). Organic compounds leaving the column are burnt in a hydrogen-oxygen flame. Ions produced are attracted to electrodes to cause a signal.

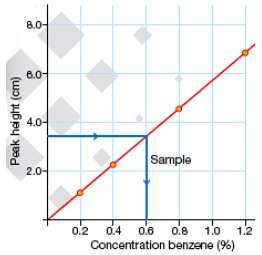
Example 6.3

The concentration of benzene in a sample of petrol was determined by gas chromatography. A series of standards with an accurately known concentration of benzene were run under the same conditions as the sample.



The areas under the peaks were measured and a calibration curves was plotted from the data.

The concentration of the sample can be read off the graph.



Chapter review

Principles of chromatography

3. Write a definition of the following terms: Adsorption, desorption, mobile phase, staionary phase, eluent, retention time, carrier gas.

4. There are several types of chromatography, including thin-layer and paper, gas and high performance liquid chromatography. What features are common to all kinds of chromatography?

Thin-layer Chromatography

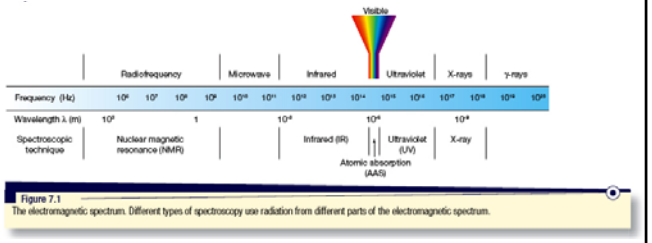
6. Phenacetin was once an ingredient in analgesic drugs, but is is not used not because it causes liver change. It is soluble in chloroform. A chemist wishes to analyse a brand of analgesic using thin-layer chromatography to determine whether it contains phenacetin. Outline the steps in the analysis.

7. A sample of brown dye from a lolly is placed at the origin on a chromatography plate. The solvent front moves 9.0 cm from the origin. A blue component of the dye moves 7.5 cm and a red component 5.2 cm in the same time. Calculate the R_f values of the two components.
   Blue 0.83 red 0.58

9. Thin-layer chromatography showed that the black dye used in a brand of writing ink contained red, blue, orange and yellow components. The R_f values of these substances using ethanol as solvent are 0.59, 0.32, 0.80, and 0.19 respectively.
   a. How far apart would the blue and yellow components be after the solvent front had moved 8.0 cm from the origin? 3.2 cm
   b. When the red component had travelled 6.0 cm from the origin, how far would the orange component have travelled? 15 cm
   c. Sketch the chromatogram of the ink to scale after the solvent front had moved 15 cm form the origin.

Spectroscopy

All forms of spectroscopy use a part of the electromagnetic spectrum to give information about substances



Radiation from each portion of the spectrum has a specific frequency, wavelength and energy associated with it.

Energy from different parts of the spectrum can be used for different spectroscopic techniques, for qualitative and quantitative analysis.

Spectroscopic techniques provide us with information about:

- The type of atom/molecule present (qualitative analysis)
- How much of a particular atom/molecule is present (quantitative analysis)
- The structure and bonding of the molecule.

The basis of spectroscopic techniques

- Atoms and molecules can absorb energies of some wavlengths and transmit energy of different wavelengths.
- By irradiating a sample with energy of different wavelengths, an absorption spectrum is produced.
- The absorption radiation may cause:
  - bonds to stretch or bend vigorously
  - electrons to jump to higher energy levels
  - nuclei to be in resonance

TABLE 7.1 Spectroscopic techniques make use of the way electromagnetic radiation interacts with atoms and molecules

Spectroscopic technique	Part of the electromagnetic spectrum	Wavelength range (cm) (approx)	Part of atom or molecule affected
Ultraviolet spectroscopy (UV)	Ultraviolet	4×10^{-5} to 10^{-7}	Electrons in molecules
Colorimetry	Visible	7×10^{-5} to 4×10^{-5}	Valence electrons in molecules
Atomic absorption (AAS) and atomic emission spectroscopy (AES); flame tests	Visible	7×10^{-5} to 4×10^{-5}	Valence electrons in atoms

Analysis of atoms

Three techniques that use radiation from the visible region of the electromagnetic spectrum: flame tests; atomic emission spectroscopy; atomic absorption spectroscopy.

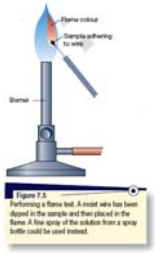


TABLE 7.2 Flame colours*

Metal	Flame colour
Sodium	Yellow
Strontium	Scarlet
Copper	Green
Barium	Yellow-green
Lithium	Crimson
Calcium	Red
Potassium	Lilac

*Some common metal ions, including iron, silver, tin, aluminium, zinc and magnesium, do not produce flame colours. They emit radiation in the **ultraviolet** (UV) region of the electromagnetic spectrum.

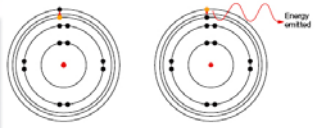


Figure 7.7 During a flame test, electrons 'jumping' from a high to a low energy level lose energy by emitting coloured light.

Atomic Emission Spectroscopy (AES)

Flame tests limited because
- Only a few elements give a colored flame
- Some colors are alike

Two improvements used in AES
- Using a hotter flame to excite electrons in a wider range of elements
- Separating emitted light through a prism to give an emission spectrum

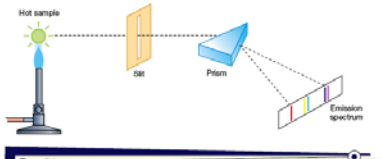


Figure 7.8 Essential elements of an atomic emission spectrometer.

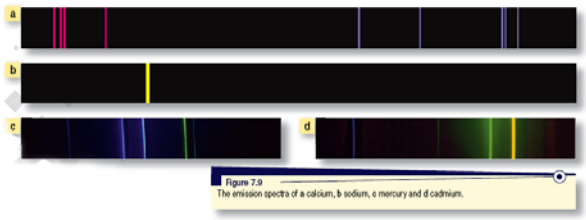


Figure 7.9
The emission spectra of a calcium, b sodium, c mercury and d cadmium.

Atomic Absorption Spectroscopy (AAS)

- AES is only useful for identifying a limited number of metals (particularly Group 1 and 2 elements) because few elements are excited even by the hottest laboratory flame.
- AAS looks at the light absorbed by atoms rather thanat the light emitted by them.
- This method is more sensitive and accurate than AES, and can be used for a much wider range of metals.
- AAS is:
  - One of the most widely used modern instrumental techniques.
  - An Australian invention used all round the world which has earned millions of export dollars for the country.
  - Very versatile: can detect over 70 elements.
  - Extremely sensitive, detecting concentrations at parts per million (ppm), or as high as parts per billion in some cases.

How AAS works

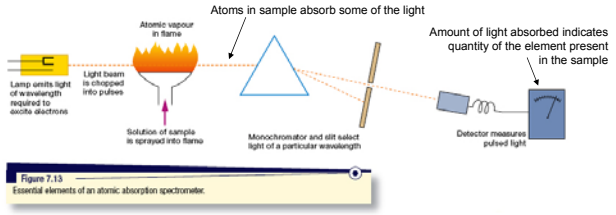


Figure 7.13
Essential elements of an atomic absorption spectrometer.

- Atoms will absorb light if the energy of the light is exactly that required to promote an electron from its normal energy level to a higher level.
- Each element has a unique absorption spectrum and requires a light source that will emit light of the correct wavelength.
- A hollow cathode lamp is made of the element of interest which when vaporised will produce light of the correct wavelength.

UV-Visible Spectroscopy

Used for determining the concentration of a substance in a sample.
A wavelength is chosen at which the substance absorbs strongly but not other components in the sample.
The absorbance of the sample is then measured at this wavelength and compared to the absorbance of standard solutions.

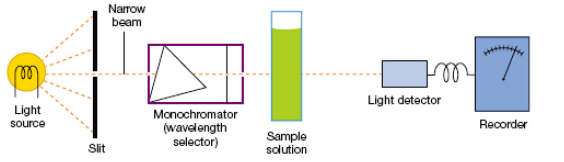


Figure 7.15
Essential elements of a simple UV–visible spectrophotometer.

Double-beam scanning spectrophotometer

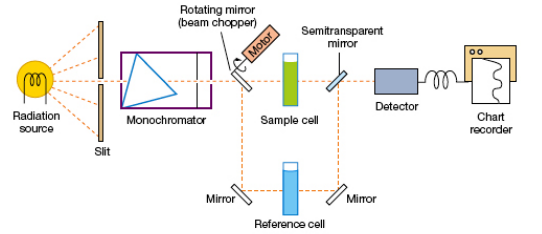


Figure 7.17
Essential elements of a double beam scanning spectrophotometer. The light beam is passed alternately through the sample and the reference cells by the rotating mirror ("beam chopper").

Chapter review

P74 Q13; 14; 15; 16; 17

P88 Q6; 7; 8; 9; 10; 12