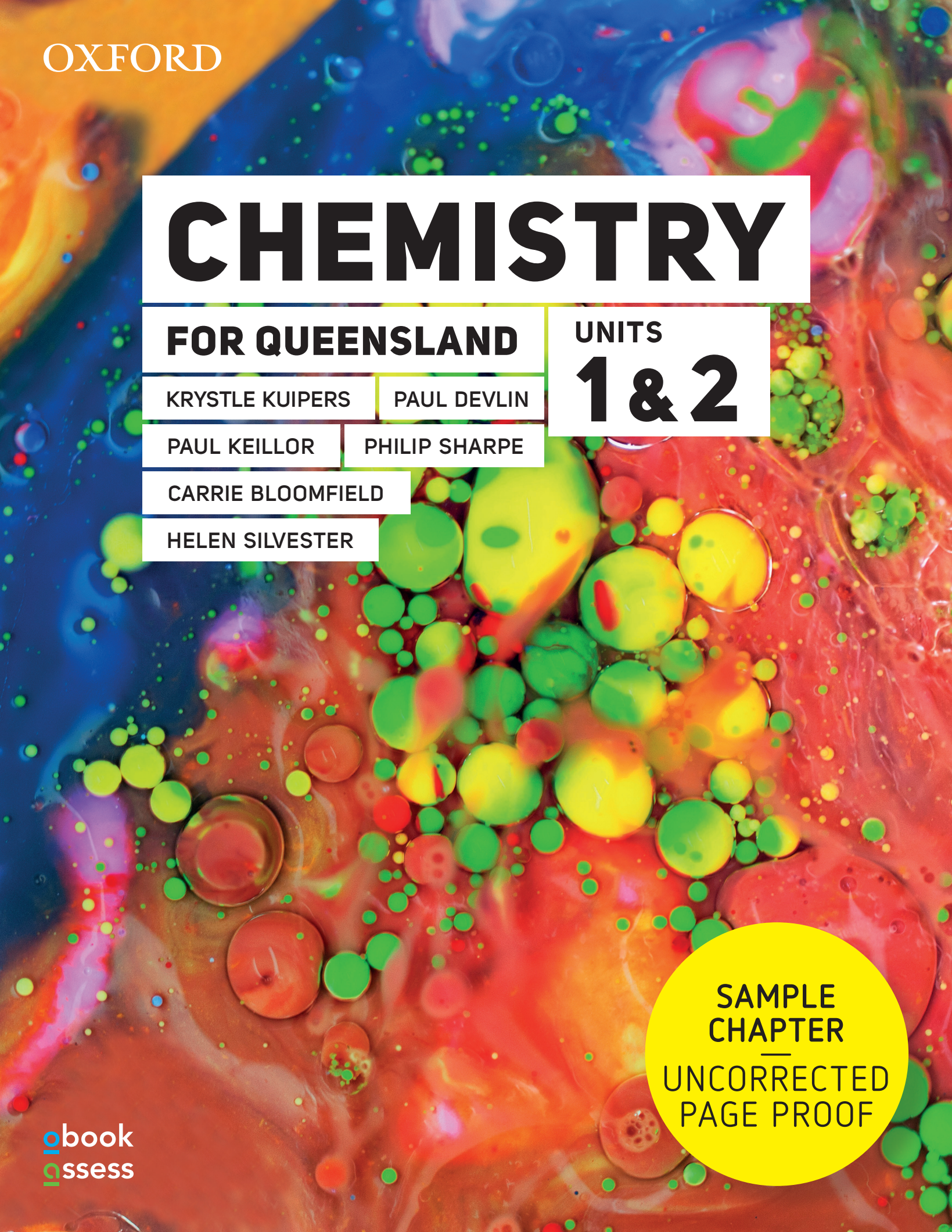
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CONTENTS

CHAPTER 13

Chromatography techniques

The word *chromatography* is derived from the Greek words for colour and write. It quite literally means 'to write with colour'. Early chromatography was based on these principles.

Chromatography is based on the broad field of separation sciences. Very simply, this field of science focuses on how to separate the chemicals contained within a mixture (Figure 1). Early chromatography was able to separate photosynthetic plant pigments into four colours: orange, yellow, blue/green and green.

Not long after this was achieved, it was discovered that chromatography could be applied to most other mixtures of chemicals, regardless of whether they were coloured or not.

Chromatography is essential within the field of chemical analysis. Today, chromatographic techniques focus on identifying the components of a mixture and determining how much of each component exists within a sample.

To fully understand the chromatographic techniques involved with this type of chemistry and the principles that underline them, it is important to have a good knowledge of the intermolecular forces covered in Chapter 12.

OBJECTIVES

- → recognise that chromatography techniques, including paper, thin-layer, gas and high-performance liquid chromatography, can be used to determine the composition and purity of substances
- → describe and explain how variations in the strength of the interactions between atoms, molecules or ions in the mobile and stationary phases can be used to separate components
- → analyse, interpret and evaluate data from chromatographs to determine the composition and purity of substances, including calculating R_f values.

PRACTICAL

SUGGESTED PRACTICAL	**13.2A** Separating the components of a mixture using paper chromatography

FIGURE 1 Chromotography separates the components of mixtures.

13.1

Principles of chromatography

KEY IDEAS

in this section, you will learn about:

- interactions between atoms, molecules or ions in the mobile and stationary phases of chromatography
- separating atoms, molecules or ions in a mixture
- describing separation processes in chromatography by using the terms 'affinity', 'adsorb' and 'desorb'.

mobile phase
the phase that flows in chromatography, moving the components of a sample at different rates over the stationary phase

solvent
a substance that can dissolve other substances; the mobile phase in chromatography because it must dissolve the sample to move it across the stationary phase

stationary phase
the phase to which the components of a chromatographic sample are adsorbed

solute
a substance dissolved in a solvent; the sample in chromatography is often a mixture of substances

affinity
the interaction of a substance within a sample with the mobile or stationary phases

adsorb
the attraction of a substance within a sample to the stationary phase

desorb
the release of a substance within a sample from the stationary phase into the mobile phase

Chromatography is an analytical technique that can be used for qualitative or quantitative analysis. It can both identify components in a mixture and determine how much of each component is present. It is a useful form of separation science that uses intermolecular forces, covered in Chapter 12, to separate the components of a mixture according to their properties.

Chromatography has a wide range of uses, including:

- pharmaceuticals – identifying elements and molecules in drugs as well as measuring the purity of medicines
- environmental science – analysing water quality and gases in the air and the harmful impact they may have on Earth
- police work – analysis such as breathalysers and drug detection
- forensic science – analysing material such as blood and hair samples
- biomedical research – analysing material such as proteins in cancer research
- food science – analysing the nutritional quality of food and food spoilage.

Stationary and mobile phases

All chromatography uses two phases to separate a mixture. These are the:

- **mobile phase**, a **solvent**, which remains in motion
- **stationary phase**, which is either a solid with a high surface area or a liquid coated onto a solid support. It always stays still.

The mobile phase moves through and carries a sample (**solute**) over the stationary phase.

Components of the sample will be more attracted to either the mobile or the stationary phase, depending on their intermolecular forces. This makes the components move at different rates and separates them. Several key terms are used to describe this attraction (Figure 1).

- **Affinity**: components of a sample are attracted, by intermolecular forces, to the mobile or stationary phase. High affinity means that the component interacts strongly with the mobile or stationary phase.
- **Adsorb**: components of the sample will adsorb onto the stationary phase from the mobile phase if they are attracted to, or have an affinity for, it.
- **Desorb**: components of the sample will desorb off the stationary phase into the mobile phase if they are attracted to, or have an affinity for, it.

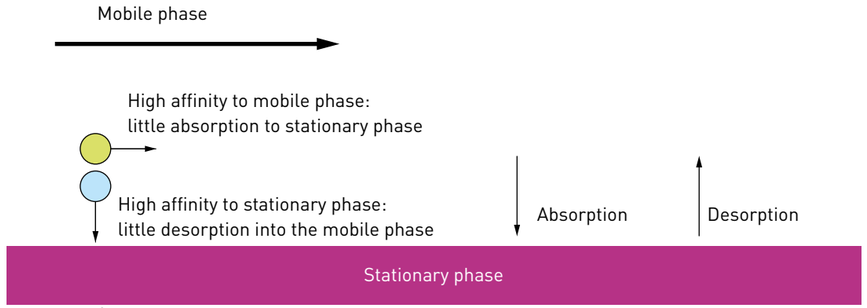


FIGURE 1 The blue and green components in this sample can be separated depending on their affinity to the mobile and stationary phases.

The 'like with like' rule

As a general rule, atoms, molecules and ions will be more attracted to the mobile or stationary phase that has the same or similar properties. Thus, like goes with like.

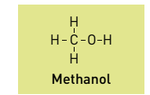
For example, ions contain one or more whole charges. Because of this, they will be more attracted to the mobile phase if it contains charged or partially charged molecules or ions, such as water (a polar molecule).

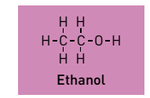
Separation based on carbon chain length/molecular mass

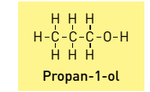
Chemists often need to separate mixtures of molecules that have the same intermolecular forces; for example, mixtures of alcohols.

In a mixture of methanol, ethanol, propan-1-ol and butan-1-ol (Figure 2), all of the molecules have the same polar –OH group on the terminal, or end, carbon. Therefore, this mixture will not be separated into its components by functional group. Rather, the mixture is separated by the size of the carbon chain – the longer the carbon chain, the greater the dispersion forces and the higher the interaction with the mobile or stationary phases.

In general, larger molecules experience more interaction and move slower because they have a greater dispersion forces between the mobile and stationary phases.







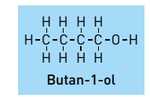


FIGURE 2 Methanol, ethanol, propan-1-ol and butan-1-ol are alcohols that have a terminal polar O–H group.

Study tip

Chromatography is based on intermolecular forces, so, when answering questions, start by determining which intermolecular forces are involved. Once you have determined the intermolecular forces, explain their affinity to the mobile or stationary phase. Then explain the effect this will have on the data you obtain (data will be covered in sections 13.2 and 13.3).

CHECK YOUR LEARNING 13.1

Describe and explain

1 Explain the following terms and why they are essential to the separation of mixtures.
   - **a** mobile phase
   - **b** stationary phase
   - **c** affinity
   - **d** intermolecular forces

Apply, analyse and interpret

2 What intermolecular forces, studied in Chapter 13, could be used to separate a mixture of substances?

3 A mobile phase primarily consists of water. Consider why water is chosen as a mobile phase.

Investigate, evaluate and communicate

4 A mobile phase chosen in an analysis contains only dispersion forces. Determine what intermolecular forces the stationary should phase have. Explain why.

5 Why do like forces attract? Assess the 'like with like' rule works.

13.2 Paper and thin-layer chromatography

KEY IDEAS

In this section, you will learn about:

- separating substances by paper and thin-layer chromatography
- calculating R_f values to identify substances within a mixture
- manipulating mobile and stationary phases to optimise separation.

paper chromatography an analytical technique for separating and identifying mixtures; the stationary phase is a thin strip of absorbent paper

thin-layer chromatography (TLC) an analytical technique for separating and identifying mixtures; the stationary phase is typically a thin layer of silica gel, aluminium oxide or cellulose supported on a piece of glass or plastic

chromatogram the pattern of bands, spots or peaks formed on the chromatography paper or TLC plate demonstrating the separation of a mixture

origin the line applied to a chromatogram to mark the point where the sample or standard was placed

solvent front the point on a chromatogram where the mobile phase reaches before the analysis is terminated

In **paper chromatography** and **thin-layer chromatography**, a sample containing a mixture of substances is applied to the stationary phase as a dot. The dot is carried up the stationary phase by the mobile phase. The resulting **chromatogram** is qualitative in nature – it can only be used to identify substances, not determine how much is present.

Paper chromatography

In paper chromatography, the stationary phase is a thin strip of absorbent paper, which is cut to fit inside a container that holds the mobile phase (Figure 1). A pencil line is ruled across the bottom of the paper to mark where the sample will be placed – this is called the **origin**. Pen or ink should not be used to mark the origin because inks will separate and contaminate the sample. Standards of known identity can also be placed on the paper to help identify the substances in the sample. In Figure 1 the standards are marked A, B and C.

The mobile phase is added to the container so that it sits *below* the origin line. The paper is then placed into the container and left to absorb the mobile phase. The sample travels up the piece of paper, separating into its components.

Components of the sample that have a strong affinity to the stationary phase move slowly. On the resulting chromatogram, they have not moved far from the origin. Components that have a higher affinity to the mobile phase move faster. On the resulting chromatogram, they have moved further and are located closer to the **solvent front** (Figure 1).

In Figure 1, the mobile phase is water and the stationary phase is filter paper. Paper is a derivative of cellulose, which contains many polar –OH groups. However, very few of the intermolecular forces of paper extend beyond its network of fibres (and any surface coating). Water is also a polar molecule, but more polar than the paper. Therefore, any component of the sample that has whole or partial charges, such as ionic or polar substances, will have

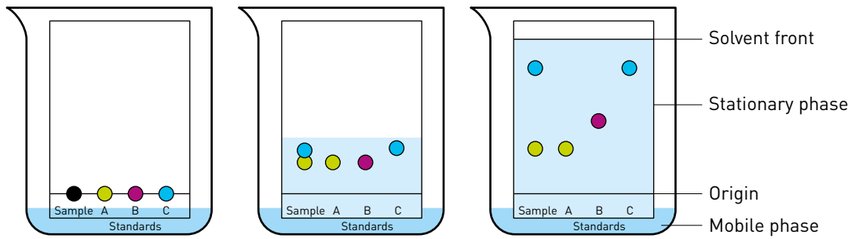


FIGURE 1 The water mobile phase moves up a piece of filter paper (stationary phase) to separate the ink in black marker pen. Components in the sample can be identified by comparing them to known standards A–C.

SUGGESTED PRACTICAL
13.2 Separating the components of a mixture using paper chromatography
Go to page xxx »

FIGURE 2 Paper chromatography can be used to separate the components of photosynthetic pigment (leaf stain). The pigment is placed on the origin, and then the paper strip is placed in a container where it is left to absorb the mobile phase.

a higher affinity, or attraction, to the water mobile phase. These components move further from the origin. Any component of the sample that experiences dispersion forces has a higher affinity to the stationary phase and will not move as far from the origin.

retardation factor (R_f) the ratio of the distance travelled by a component of a sample, from the origin, to the distance travelled by the mobile phase

Retardation factor (R_f) calculations

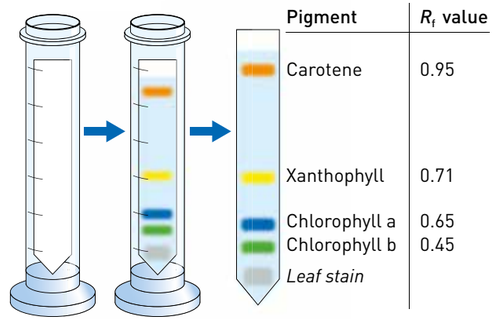
Once the separation is completed, the resulting chromatogram is analysed to identify the substances in the sample and how pure the sample is (composition and purity). Although we can simply look at a chromatogram to judge whether two substances are identical, it is more precise to calculate the **retardation factor (R_f)** of a substance. This is the ratio of the distance moved by a substance to the distance moved by the solvent, or mobile phase.

This can be simplified to:

$$R_f = \frac{\text{distance solute moves from origin}}{\text{distance solvent solvent moves from origin}}$$

$$= \frac{\text{distance of sample dot}}{\text{distance of mobile phase}}$$

Each atom, ion or molecule has a unique R_f depending on the properties of the mobile and stationary phases. Increasing the polarity of the mobile phase increases its affinity to charged particles within the sample. This makes the substances move further up the paper.



Pigment	R_f value
Carotene	0.95
Xanthophyll	0.71
Chlorophyll a	0.65
Chlorophyll b	0.45
Leaf stain	

FIGURE 3 Separation of photosynthetic pigments into its constituent pigments. The R_f value is calculated from its chromatogram.

Study tip

Never leave R_f as a fraction. This is because it is analysed on a scale of 0–1, with 0 being no affinity to the mobile phase and 1 being no affinity to the stationary phase.

All R_f values must be expressed as a decimal (never a fraction) and cannot be greater than or equal to one. An R_f of 1 indicates that the substance has not separated from the mobile phase and therefore cannot be identified. The analysis must be run again under different mobile or stationary phase conditions.

Thin-layer chromatography

Thin-layer chromatography (TLC) works on the same principles as paper chromatography with one major change that makes the process more efficient (faster and more sensitive). In TLC, the support for the stationary phase is a piece of glass or plastic, which is coated in the stationary phase, consisting of silica gel, aluminium oxide or cellulose. As this coating is a thin layer spread on the surface of the plastic or glass, the technique was named 'thin-layer' chromatography.

Study tip

Both paper and thin-layer chromatography are named after the stationary phase. This may help with recalling specific information about these techniques.

The components within the sample are separated, as with paper chromatography, according to their affinity to the mobile or stationary phase.

It is important to note that both paper chromatography and TLC are not limited to coloured compounds. Fluorescent TLC plates can be used under UV light to visualise the components of a sample that would not otherwise be visible. These are shown in the darker areas of the plate where the sample blocks the fluorescence of the plate (Figure 4).

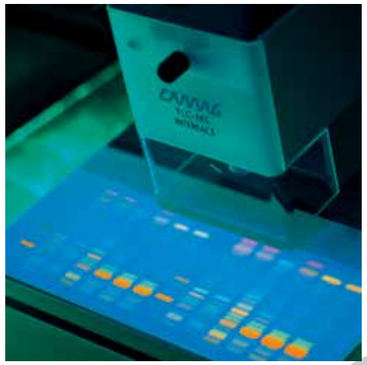


FIGURE 4 TLC performed on fluorescent plates.

WORKED EXAMPLE 13.2

A food dye was analysed against three standard dyes A–C to determine which ones were present. The resultant chromatogram is shown in Figure 5.

Use the data to determine:

a the retardation factors of the dye standards
b the retardation factors of all dyes within the sample
c the dye that is more strongly attracted to the stationary phase
d the dye that is more strongly attracted to the mobile phase
e which dyes are in the sample
f whether the analysis of this data valid.

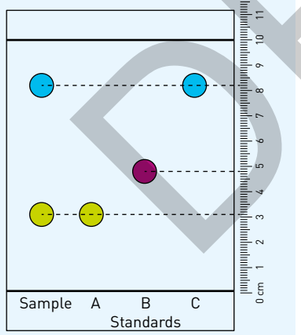


FIGURE 5 The chromatogram from analysing food dye against three standard dyes.

Solution

a Table 1

Green dye A	Purple dye B	Blue dye C
$R_f = \frac{3.1}{10} = 0.31$	$R_f = \frac{4.8}{10} = 0.48$	$R_f = \frac{8.2}{10} = 0.82$

b Table 2

Green dye A	Blue dye C
$R_f = \frac{3.1}{10} = 0.31$	$R_f = \frac{8.2}{10} = 0.82$

c Green dye A has the highest affinity to the stationary phase. It has a higher rate of adsorption to the stationary phase because it has moved less distance from the origin.
d Blue dye C has the highest affinity to the mobile phase. It has a higher rate of desorption from the stationary phase because it has moved further from the origin.
e The green dye in the sample and standard A have the same R_f of 0.31. The blue dye in the sample and standard C both have an R_f of 0.82.
f All R_f values are less than 1. This means that all of the substances have separated from the mixture and the data is valid.

Two-dimensional paper or thin-layer chromatography

Occasionally, a chromatography technique will not separate some components sufficiently within the sample. This is because the components have the same amount of interaction with the mobile and stationary phases. Rather than repeating the analysis, chemists will rotate the chromatogram by 90°, so that the sample is on the bottom. They rule a new origin line and run the analysis again, using a different mobile phase with different properties (Figure 6). The chromatogram is analysed by using R_f calculations for analysis 1 and also for analysis 2.

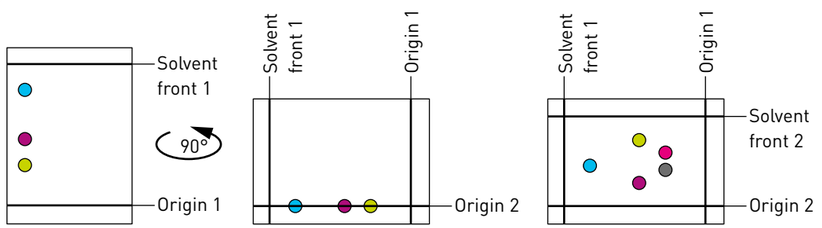


FIGURE 6 Two-dimensional paper chromatography. After the first analysis, the chromatogram is rotated 90°. This gives a new origin line. The second chromatography analysis separates the green and purple dots further into their individual components. (Origin 1 and solvent front 1 refer to the first analysis. Origin 2 and solvent front 2 refer to the second analysis.)

Study tip

If $R_f = 1.0$, then no separation from the stationary phase has occurred. A typical exam question may ask how to separate this component in a further analysis. Look at the properties of the stationary phase and determine what you could do to make this substance more attracted to it.

CHALLENGE 13.2

Improving separations

A chemist performs a separation under the following conditions.

» Mobile phase: water and 10% ethanol
» Stationary phase: paper

1 What conclusions can you make about the properties of the components of the sample?
2 What alterations would you make to the mobile phase to obtain a better separation of the components?

CASE STUDY 13.2

Separation of amino acids in proteins

Proteins are synthesised by the body when many amino acids are joined into long chains of amino acid residues. Each protein synthesised by the body has a unique function depending on the number and sequence of amino acids in the chain.

In the early 1900s, biochemists were looking for a way to determine the number and sequence of amino acids in proteins. They were largely unsuccessful because of the structure of the amino acid molecules. Amino acids have the same general structure (Figure 7). The only difference between amino acids is the structure of the R group, which gives rise to the different properties of each amino acid and therefore the function of each protein.

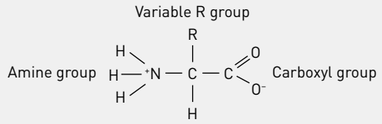


FIGURE 7 The structure of an amino acid.

The problem of separating amino acids was overcome in 1941 when paper chromatography was first developed by British chemist Archer J.P. Martin and biochemist Richard L.M. Synge. Martin and Synge stained the amino acid residues with ninhydrin, a purple dye, and used a piece of filter paper dipped in water as the stationary phase with butan-1-ol as the mobile phase. The characteristic properties of the R group alter the solubility of the amino acid in the mobile phase. Therefore, each amino acid in a protein can be separated by chromatography.

However, some amino acids with similar R groups did not separate. The scientists then decided to rotate the paper by 90° and run it again with a different mobile phase. Thus, not only paper chromatography, but two-dimensional paper chromatography, was born.

Martin and Synge's research became popular throughout the late 1940s and 1950s with major developments in biochemistry being made by using paper chromatography. British chemist Frederick Sanger used this research to determine the sequence of amino acids in insulin, a biochemical that is essential to blood sugar regulation within the body.

Martin and Synge won a Nobel Prize for their work in 1952.

CHECK YOUR LEARNING 13.2

Describe and explain

1 Describe, making reference to Figure 6, the steps involved in calculating retardation factor.

2 Explain how components of a mixture are identified by paper or thin-layer chromatography.

Apply, analyse and interpret

3 Analyse the chromatogram in Figure 8.
- **a** Calculate the R_f values for all components.
- **b** Which component has the highest affinity to the mobile phase? Explain why.
- **c** Which component has the highest affinity to the stationary phase? Explain why.

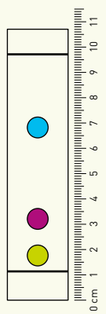


FIGURE 8 A chromatogram.

4 If the chromatogram were run with a polar stationary phase, explain the types of intermolecular bonding that may be present between each component and the stationary and mobile phases.

Investigate, evaluate and communicate

5 The R_f values of 10 food dyes are shown in Table 3. These R_f values are specific for TLC using a silica plate and a 1% ethanol mobile phase.

TABLE 3 R_f values of some food dyes

Dye	R_f
Brilliant blue FCF	0.12
Indigotine	0.23
Fast green FCF	0.28
Erythrosine	0.45
Quinoline Yellow	0.50
Carmoisine	0.60
Tartazine	0.65
Green S	0.72
Patent blue V	0.86
Ponceau 4R	0.91

Three foods A–C were tested to determine which food dyes were present. The resultant chromatogram is shown in Figure 9.

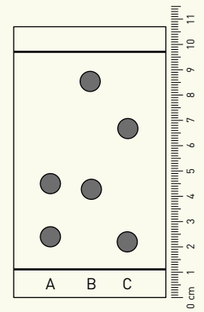


FIGURE 9 A chromatogram of food dyes.
- **a** Calculate the R_f values of the components of each sample and determine which food colourings were present in the foods.
- **b** There are two components of these samples that, upon visual inspection, appear to be the same. Are these components the same? Explain your answer

6 A crime scene note was written in black pen. Two black pens found at the scene were analysed by forensic scientists to determine which pen had written the note. To ensure valid results, five teams were given one of the two pens to test. Each team developed a method that involved using paper chromatography with a water mobile phase. The data in Table 4 was obtained.

TABLE 4

	Distance (cm) travelled by:			
Team	Solvent front	Component 1	Component 2	Component 3
1	11	6.16	8.69	2.31
2	15	4.80	8.85	13.05
3	9	5.04	7.11	1.89
4	21	11.76	16.59	4.41
5	18	5.76	10.62	15.66
Crime scene note	25	8.00	14.75	21.75

- **a** Use the data to calculate the R_f values of components 1–3.
- **b** Which of the teams analysed the same pens?
- **c** Which teams analysed the pen that was responsible for the note at the crime scene?
- **d** Is component 1 the same substance for both pens? Explain your answer.
- **e** Is it possible to identify the components of the sample from this analysis?

Check your obook assess for these additional resources and more:

» Student book questions

» Weblink – Suggestion: as per Syllabus, simulation of a TLC

» Weblink –

13.3

Column chromatography

KEY IDEAS

In this section, you will learn about:

- the processes involved with separating mixtures by column chromatography
- interpreting gas and high-performance liquid chromatograms
- determining retention times (R_t) to identify substances
- determining concentration using calibration curves.

column chromatography
a technique used to separate and purify individual components from mixtures of compounds

column
the tube-like structure that contains the stationary phase in column chromatography and through which the mobile phase and sample flow

Column chromatography is a more advanced form of separation science. It uses the same basic principles of paper and thin-layer chromatography, but the stationary phase is contained within a tube-like structure.

The simplest form of column chromatography (Figure 1) uses a glass **column**, similar to a burette, which contains a stationary phase of aluminium oxide or silica. The stationary phase resembles finely ground sand. The stationary phase must be packed tightly to minimise gaps. Otherwise, the sample will move too fast and will not interact with the stationary phase. This means that less separation will occur.

FIGURE 1 Column chromatography in a laboratory.

The mobile phase is poured into the top of the column and allowed to run slowly down through the stationary phase until it reaches the tap at the bottom. Once the column is soaked in the mobile phase, the sample is added to the top of the column and the tap at the bottom is opened. This allows the mobile phase to run through the column with the sample.

The sample separates into its various components depending on their affinity to the mobile or stationary phase. A higher affinity to the mobile phase means that a component spends longer interacting with it. As the mobile phase flows through the column, the component moves with it and it is collected earlier (Figure 2).

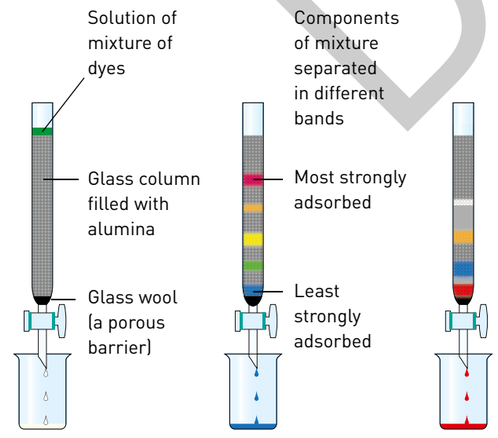


FIGURE 2 Separation of the components of a sample.

The various components of the sample are collected in separate beakers as they **elute**, or come out of, the column. Once the components are separated, further techniques are used to identify and quantify them.

There are several types of column chromatography. The two most commonly used are **gas chromatography** and **high-performance liquid chromatography (HPLC)**.

Gas chromatography

In gas chromatography, the mobile phase is an inert gas (carrier gas), such as nitrogen (N_2). The sample is **vaporised** and injected into the mobile phase, which carries the sample through a very small (1–2 mm diameter) and very long (up to 60 m or longer) column containing the stationary phase. The stationary phase can be either a solid, packed into the column, or a liquid that coats the inside of the column (Figure 3). Both stationary phases must be able to withstand very high temperatures because the column is contained within an oven to ensure that the sample remains vaporised throughout the separation.

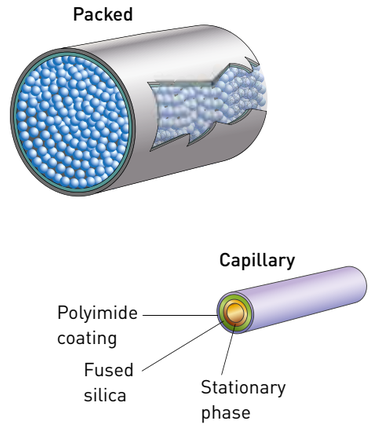


FIGURE 3 The two types of columns used in column chromatography. One is a tube packed with small particles; the other is a much smaller capillary-sized tube, which has a coating on the inside.

The sample separates as each component interacts with the stationary phase. There is no attraction to the mobile phase because it in an inert gas; its purpose is to move the sample through the column. The components with a low affinity to the stationary phase elute from the column first – they are not retained on the column for long. The components with a high affinity to the stationary phase are retained on the column for a longer period of time.

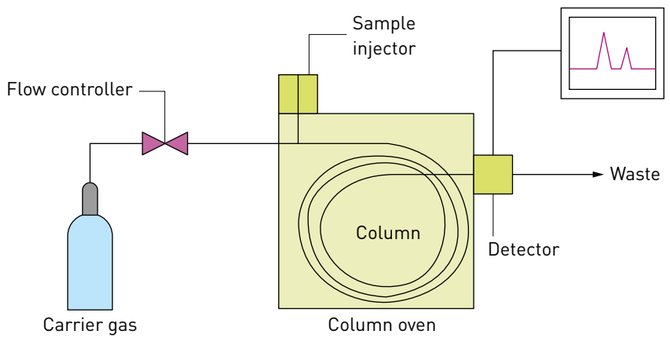


FIGURE 4 A simplified diagram of a gas chromatograph.

The resulting chromatogram is obtained when the detector recognises the components as they elute from the column. Each component forms a peak in the chromatogram as it elutes (Figure 4).

elute
come out of the bottom of a chromatography column

gas chromatography
a technique used to separate and purify individual components from mixtures of compounds that can be vaporised

high-performance liquid chromatography (HPLC)
a technique used to separate and purify individual components from mixtures of compounds by pumping a pressurised liquid containing the sample through a column of adsorbent material

vaporise
to become gaseous

Interpreting a gas chromatogram

Figure 5 depicts a gas chromatogram that has been obtained from the separation of a mixture of alcohols. The detector is started at time zero, when the mixture is injected into the column. At 39 seconds, methanol has eluted from the column. Therefore, methanol must have the lowest affinity to the stationary phase. The chromatogram ends with hexanol, which elutes from the column at 162 seconds. Hexanol has the highest affinity with the stationary phase, interacting more with it and eluting last.

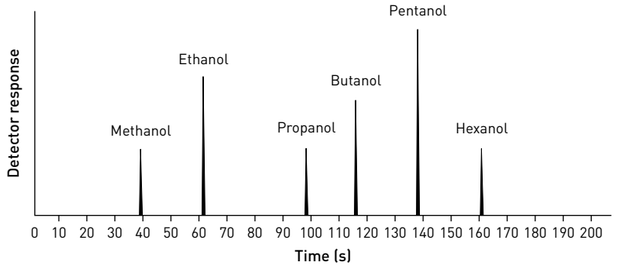


FIGURE 5 A gas chromatogram obtained from the separation of a mixture of alcohols.

TABLE 1 Retention times of components of a mixture of alcohols in Figure 5

Component of the sample	R_t (s)
Methanol	39
Ethanol	63
Propanol	98
Butanol	115
Pentanol	138
Hexanol	162

Retention time

retention time (R_t) the time that each component is retained by the column

The time that it takes a sample to move through the column, from injection to detection, is called the **retention time (R_t)**. It is the time that each component is retained by the column. Thus, it indicates the amount of interaction that has occurred between each component of the sample and the stationary phase. Table 1 represents the retention times for the components within the alcohol separation of Figure 5. Figure 6 shows how to measure R_t values.

Study tip

Do not confuse R_t with R_f. In column chromatography, a low R_t means the substance has travelled through the column fast and has a high affinity to the mobile phase. In paper or thin-layer chromatography, a low R_f means the substance has travelled up the stationary phase slowly and has a high affinity to the stationary phase.

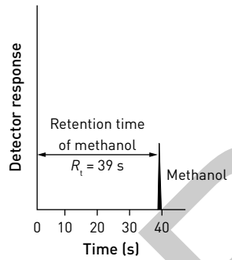


FIGURE 6 The retention time is the amount of time that a component is retained by the column.

Identifying components

As with paper chromatography and TLC, standards are used in gas chromatography to identify the components within a mixture qualitatively. Figure 7 shows a chromatogram of a propanol standard run under the same conditions as the chromatogram in Figure 5.

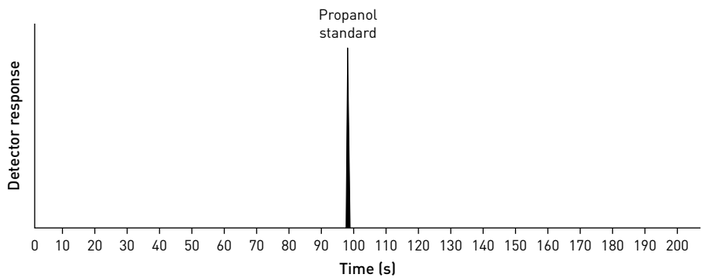


FIGURE 7 A gas chromatogram of a propanol standard.

Propanol has an R_t of 98 seconds. If the standards are run under the same conditions as the sample, they will have identical retention times to the components of the sample. Therefore, standards that are run under the same conditions can be used to identify components of a sample.

Concentration of components

Column chromatography has the advantage of measuring the components of a sample quantitatively. This is done by measuring the height of the peak or the area of the peak. Fortunately, most chromatographs are computerised and programmed to complete this calculation. A computerised HPLC system will usually measure peak area rather than height. The *y*-axis of the chromatogram measures peak area in cm^2. The greater peak height is due to more of that compound eluting over the same very short interval.

Figure 8 demonstrates how this technique is performed. In this example, five pure samples of ethanol were run on a gas chromatograph under identical conditions. Each sample had an accurately known concentration. A sample of ethanol of unknown concentration was also run. As well as the resultant chromatograms, a table of peak area data was generated (Table 2).

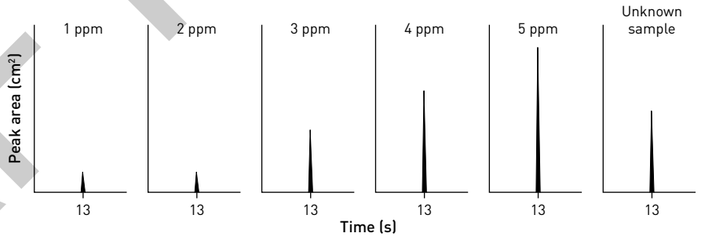


FIGURE 8 The chromatograms of five standards of ethanol and an unknown sample.

TABLE 2 Peak areas of ethanol standards and an unknown sample in Figure 8

Sample	Concentration (ppm)	Peak area (cm^2)
Standard 1	1	5
Standard 2	2	10
Standard 3	3	15
Standard 4	4	20
Standard 5	5	25
Unknown sample	–	19

calibration curve a graph of peak area against concentration of a set of standards from a chromatogram

A graph can be constructed from the data in Table 2, using only the standards (Figure 9). This plots peak area against concentration.

The graph demonstrates a relationship between peak area and the concentration of the standards. This is called a **calibration curve**. If the peak area of the unknown sample is within the range of the standards (Table 2), the calibration curve can be used to determine its concentration. Figure 9 shows that a peak area of 19 corresponds to a concentration of 3.8 ppm. This can be determined by ruling lines to the linear calibration line (shown as purple dashed lines on the graph).

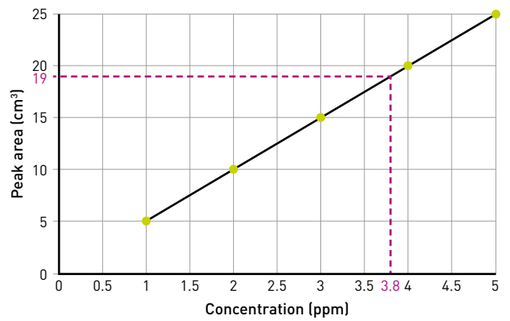


FIGURE 9 The calibration curve of a set of five standards of ethanol.

WORKED EXAMPLE 13.3

An athlete's urine was tested for a specific drug. Initial testing determined that there was some of this drug in the athlete's system, so HPLC was used to determine its concentration. The data in Table 3 was obtained.

TABLE 3 Peak areas of drug standards and an unknown sample

Sample	Concentration (mg L^{-1})	Peak area (cm^2)
Standard 1	7	0.6
Standard 2	14	1.5
Standard 3	21	2.4
Standard 4	28	3.3
Standard 5	35	4.2
Unknown	–	2.9

a Create a calibration curve for the data.

b Determine the concentration of the drug in the urine sample.

Solution

a

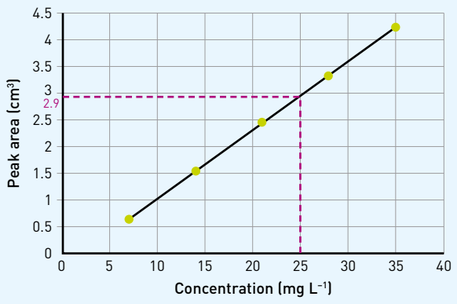


FIGURE 10 The calibration curve of a set of five standards of a drug.

b The concentration of the drug in the urine sample is 25 mg L^{-1}.

Study tip

Remember that the position of the peaks on a chromatogram is what is used to identify the components in a sample. Their peak area is used to determine how much (concentration) of the component is in the sample.

Problems with chromatograms

Sometimes, when a sample is run through a chromatograph, the first chromatogram includes peaks that overlap. This may make it impossible to measure retention time. Figure 11 shows a chromatogram with two overlapping peaks. The tops of the peaks can be easily observed, so retention time could be measured, even though the most accurate results are gained when there is complete separation of the peaks. However, the peak area cannot be measured because not all of the peak can be observed.

To avoid overlapping peaks in a chromatogram, a chemist can change the material in the stationary phase, the flow rate of the mobile phase or the temperature of the column.

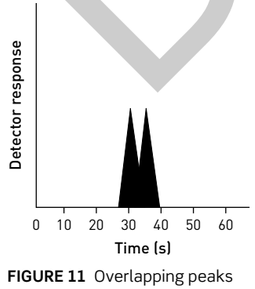


FIGURE 11 Overlapping peaks on a chromatogram can make it impossible to measure retention time and peak area.

High-performance liquid chromatography

HPLC operates on the same principles as other chromatographic techniques. HPLC uses a liquid or aqueous mobile phase, which is pumped through an instrument (Figure 12). The sample is injected, and the mobile phase moves the sample through the column at a specified flow rate. This flow rate can be altered to pump the mobile phase with the sample through faster or slower.

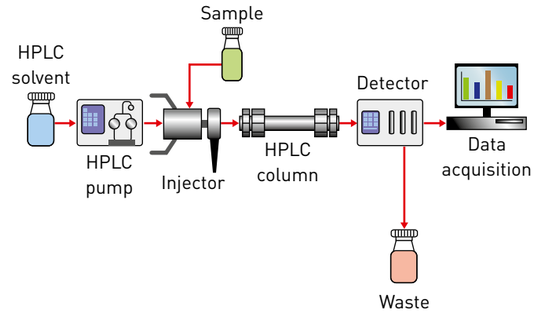


FIGURE 12 A simplified diagram of a high-performance liquid chromatograph.

The column in HPLC is a solid with a high surface area, packed at very high pressure (about 2700 kPa) into a plastic piece of tubing. It is very similar to column chromatography, but particles are much smaller and more tightly packed. As the sample moves through the column, it interacts with both the mobile and stationary phases to separate into its various components.

The detector recognises the components as they elute from the column and generates a chromatogram similar to that generated by gas chromatography.

The temperature of the column can be altered, but not to the extent of a gas chromatograph, because the column is made of plastic, which melts at too high a temperature.

Interpreting a high-performance liquid chromatogram

High-performance liquid chromatograms are analysed in the same way as gas chromatograms are. The peaks are identified by running standards under identical conditions and analysing retention times. Concentration is determined by using a set of standards of accurately known concentration, plotting a calibration curve and measuring an unknown sample on the calibration curve.

Study tip

The syllabus states that knowledge of specific aspects of the instrumentation is not required.

CHALLENGE 13.3

Overlapping peaks on a high-performance liquid chromatogram

A chemist runs a sample through a high-performance liquid chromatograph under the following conditions.

- Flow rate: 5 mL s^{-1}
- Sample concentration: both substances are 10 ppm
- Mobile phase: highly non-polar
- Stationary phase: highly polar

The chromatogram shown in Figure 13 is obtained.

1. Is this data valid? Explain why or why not.
2. What could be done to improve the separation?
3. Which analytical technique, outlined in Chapter 5, could be used to confirm the identity of the components within the mixture once they have been separated?

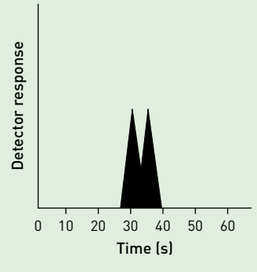


FIGURE 13 The chromatogram run by the chemist.

CASE STUDY 13.3

The horsemeat scandal of 2013

In 2013, one of the biggest food scandals across Europe was uncovered. Routine food testing in British and Irish supermarkets revealed horse DNA in beef burgers supplied by the ABP Food Group.

HPLC combined with mass spectrometry was used to analyse DNA in the burgers, which were compared with regular beef products. Scientists found inconsistencies between the burgers and the regular beef products. Of the beef products tested, 37% contained horse DNA and 85% contained pig DNA.

Although these meats are not harmful to consumers and no health issues were raised, it created major problems of trust for consumers because the products did not match the claims made on the product labelling.

HPLC showed that some burgers or other meat products contained 100% horse meat, whereas other tests revealed it to be present in only small percentages.

The ABP Food Group supplied beef patties to Burger King, Tesco, Lidl and Aldi. The scandal caused nearly 10 million beef patties to be recalled and removed from supermarket shelves while Burger King immediately announced a change in their supplier.

FIGURE 14 In 2013, HPLC was used to show that some beef burgers in the UK contained horse meat.

CHECK YOUR LEARNING 13.3

Describe and explain

1 Describe the steps involved in the creation of a calibration curve.

2 Explain the differences between the mobile and stationary phases in gas chromatography and HPLC.

3 Explain how the interaction of the components within a sample with the mobile phase and stationary phase differs in gas chromatography and HPLC.

Apply, analyse and interpret

4 A sample of coffee was analysed by HPLC to determine the amount of caffeine present. The data in Table 4 was obtained.

- **a** Construct a calibration curve for the data.
- **b** Determine the concentration of caffeine in the sample.
- **c** Consider if gas chromatography could be used to analyse the same sample.

TABLE 4 Peak areas of caffeine standards and an unknown sample

Sample	Concentration ($mg\ L^{-1}$)	Peak area (cm^2)
Standard 1	4	0.10
Standard 2	8	0.18
Standard 3	12	0.26
Standard 4	16	0.34
Standard 5	20	0.42
Unknown	–	0.23

5 An alkane is an organic molecule that contains only carbon and hydrogen atoms joined single bonds. A sample of five alkanes is run through a gas chromatograph. The stationary phase is non-polar. Table 5 outlines the number of carbons in each alkane's chain.

- **a** Explain the intermolecular forces between the alkanes and the stationary phase.

TABLE 5 Number of carbons in some alkanes

Alkane	Number of carbons
Ethane	2
Butane	4
Pentane	5
Heptane	7
Decane	10

- **b** Which alkane would you expect to elute first from the column? Explain why.
- **c** Which alkane would you expect to elute last? Explain why.
- **d** Draw a chromatogram of the resulting separation of molecules.

Investigate, evaluate and communicate

6 A calibration curve is developed to measure the ethanol content in a bottle of wine. The resulting graph is shown in Figure 15.

- **a** The sample generates a peak area of 30 cm^2. Determine the concentration of the sample.
- **b** Is this concentration valid? Explain why.
- **c** Discuss what would need to be done to improve the analysis.

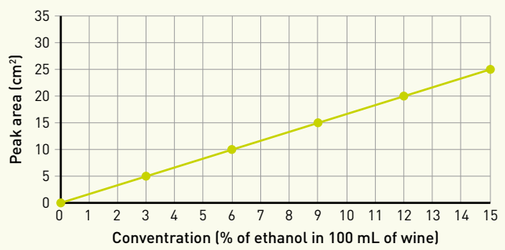


FIGURE 15 A calibration curve for ethanol in wine.

7 A woman was breathalysed to determine whether her blood alcohol reading was within a safe range to drive. A breathalyser measures the amount of alcohol (ethanol) present in your body in g/100 mL of blood.

The woman was asked to blow into the breathalyser when she was pulled over, in her car. Her initial test gave a blood alcohol reading of 0.052. Because she blew over 0.05, she was taken into the police blood alcohol testing van for a second test.

The second test gave a blood alcohol reading of 0.049, and she was released to drive home.

- **a** Discuss why the first reading was not used to charge the woman for drink driving.
- **b** What is the difference between the first and second tests?
- **c** Propose two reasons to explain why the woman's blood alcohol reading lowered between being tested in her car and being tested in the van.
- **d** Discuss is the difference between a qualitative and quantitative breathalyser test. Who would you give a qualitative test to and why would you choose it over a quantitative test?

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» Weblink –

SCIENCE AS A HUMAN ENDEAVOUR

13.4 Applications of chromatography in chemical industry

KEY IDEAS

In this section, you will learn about:

+ various applications of chromatographic techniques within the field of chemistry
+ the types of substances analysed by each chromatographic technique.

Chromatographic techniques have a wide variety of uses in the chemical industry. Primarily, these are based around forensic chemistry, food science and pharmaceuticals.

Applications of chromatography

The type of chromatography technique chosen to separate, identify and quantify a substance depends on the properties of the sample. It also depends on the limitations of each technique in analysing various substances.

Paper chromatography and thin-layer chromatography

Paper chromatography is the crudest of all chromatographic techniques. It is cheap because it requires no specialised equipment and is more commonly used for samples of compounds that can be separated by water (a polar molecule). Therefore, it is used for separating molecules by their polarity.

TLC is more sensitive (smaller concentrations of sample can be used) and efficient (faster) than paper chromatography. However, the two techniques analyse similar types of mixtures.

Common analyses include:

- determining the types of food colourings present in inks, dyes and additives in food chemistry
- determining the amino acids in proteins, for various biochemical and medicinal applications; for example, cancer research
- identifying the reactants and products in a reaction system, to determine how far a reaction has proceeded in drug synthesis
- identifying plant extracts, which may be used to develop various pharmaceutical drugs
- determining the purity of a substance; for example, pharmaceutical drugs.

Gas chromatography

Substances analysed by gas chromatography must be volatile (i.e. easily evaporated into the gas phase). Non-polar substances, or molecules with only dispersion forces, are more volatile and therefore tend to be analysed by gas chromatography rather than HPLC.

Gas chromatography is limited by the set-up of the instrument and its requirement for vaporised samples.

- Gas chromatography will only analyse volatile compounds; it cannot analyse semivolatile or non-volatile compounds.
- The instrument must be calibrated regularly because of its high sensitivity.
- Gas chromatography can typically analyse samples with a concentration in mg L^{-1} (or **ppm**). Some gas chromatographs can analyse at lower concentrations, but this is not common.
- Water vapour in the atmosphere can contaminate the analysis.
- The detector is located outside the oven that contains the column. The variation in temperature experienced by the samples moving from the column to the detector can cause errors.
- The instrument must be attached to a gas cylinder, which dispenses gas to act as the mobile phase. However, the cylinder is capable of storing large quantities of gas, so this is not often a problem.

ppm
parts per million; a unit of concentration that measures the amount of substance in milligrams per litre

Applications of gas chromatography include:

- determining the alcohol content in blood
- determining air quality; for example, the amount of gases released as pollutants by cars, power plants and various other fossil fuel burners
- determining the nutritional content of foods; for example, fatty acids, proteins, carbohydrates
- analysing pesticides that may have leached into soils, waterways and ecosystems
- identifying and quantifying cancer-causing biomolecules
- separating mixtures so that individual components can be collected and further analysed by other analytical techniques.

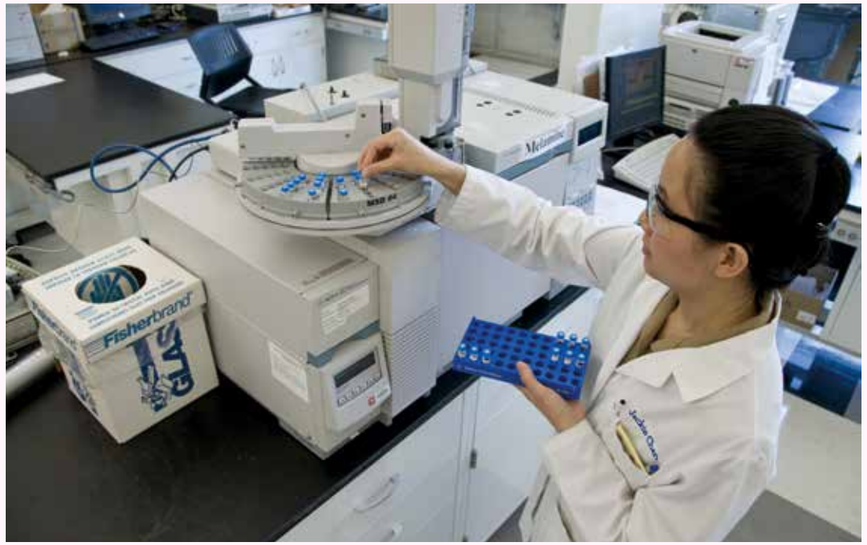

FIGURE 1 Gas chromatography can be used to test the nutritional quality of food.

High-performance liquid chromatography

HPLC is used to analyse non-volatile molecules. There are many types of HPLC that can separate mixtures according to size, charge, molar mass and even hydrophilic properties.

HPLC is limited by the set-up of the instrument and its requirement for liquid or aqueous samples.

- HPLC will only analyse compounds that are soluble in the liquid or aqueous mobile phase. HPLC cannot analyse gaseous or insoluble compounds, which can damage or block the instrument.
- The instrument must be calibrated regularly because of its high sensitivity.
- HPLC can typically analyse samples with a concentration in μg L^{-1} (or **ppb**). Some high-performance chromatographs can analyse lower concentrations but it is not common.
- The mobile phase must be kept clean to prevent impurities contaminating the sample.
- The column must be cleaned by flushing thoroughly with the mobile phase to ensure that no substances are stuck to the stationary phase, blocking its interaction with other molecules.
- The small particle size of the column results in significant resistance to the movement of the mobile phase. Therefore, the mobile phase must be pumped through at very high pressures (sometimes more than 48 000 kPa).
- The instrument must be attached to an eluent reservoir (i.e. the bottle that stores the mobile phase). This dispenses the mobile phase into the instrument. Depending on the rate that the mobile phase is pumped through the instrument and the size of the reservoir, it must be topped up frequently to ensure that the HPLC does not run out of mobile phase.

ppb
parts per billion; a unit of concentration that measures the amount of substance in micrograms per litre

Study tip

When answering questions about chromatographic technique, if possible determine the properties of the sample first. Otherwise, look for the intermolecular properties in the analysis and explain interactions and applications on the basis of these.

FIGURE 2 A scientist working with high-performance liquid chromatography checks a sample.

Applications of HPLC include:

- analysing the purity of various drugs in pharmaceutical manufacturing
- determining and quantifying illicit drugs, for use in charging and convicting persons of interest
- analysing food and blood samples to determine the presence and concentration of various nutrients
- immunoassays, which determine the presence and concentration of various molecules within the blood using antibodies
- identifying and quantifying substances in post-blast bomb residues.

CHECK YOUR LEARNING 13.4

Describe and explain

1 Describe the main differences between samples analysed by gas and liquid chromatography.

2 Explain whether samples that are analysed by TLC can also be analysed by paper chromatography.

Apply, analyse and interpret

3 Determine which analytical techniques should be used to separate or analyse the following samples.
   - **a** Drug content in blood
   - **b** Types of amino acids in a protein
   - **c** Alcohol content in wine

4 For each of the following three analyses, deduce which technique would be used to analyse the sample and why it was chosen.
   - Analysis of a crude oil sample to determine the compounds present and their concentrations
   - Analysis of a water sample to determine the compounds present
   - Analysis of a brown Smartie to determine the food colourings present

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CHAPTER 13

Review

Chapter summary

13.1
- All chromatography is based on intermolecular bonding. The type of intermolecular bonding determines how strongly the components of a mixture interact with chemicals in the mobile and stationary phases and therefore how effectively they separate.
- Components of the sample with a high affinity to the mobile or stationary phase interact more with it. Components with a lower affinity to the mobile or stationary phase do not interact as much.
- The components of a sample separate when they interact with the mobile or stationary phase as they adsorb to the stationary phase and desorb into the mobile phase.

13.2
- Paper and thin-layer chromatography are the simplest techniques for separating a sample. The resultant chromatogram is analysed by calculating the retardation factor (R_f). This determines the ratio of the movement by components of the sample to the movement of the mobile phase. Both techniques are qualitative.

$$R_f = \frac{\text{distance solute moves from origin}}{\text{distance solvent moves from origin}}$$

13.3
- Gas chromatography and HPLC involve a column that is packed with a stationary phase. The sample and mobile phase are pumped through the column. In gas chromatography, the sample interacts with the stationary phase. In HPLC, the sample interacts with the stationary phase and mobile phase. This interaction separates the sample into its components. The resultant chromatogram displays peaks, with one peak for each component of the sample.
- The chromatogram is analysed in two ways.
  - The retention time (time is has spent on the column) identifies each component qualitatively.
  - The area under the peaks measures the concentration (quantitative) of each component when analysed on a calibration curve.

13.4
- Chromatographic techniques have many applications. To determine the best technique to use, it is important to consider the properties of the components within the sample as well as the limitations and capabilities of each instrument. The mobile and stationary phases must be selected carefully according to the properties of the sample.

Key terms

- adsorb
- affinity
- calibration curve
- chromatogram
- column
- column chromatography
- desorb
- elute
- gas chromatography
- high-performance liquid chromatography (HPLC)
- mobile phase
- origin
- paper chromatography
- ppb
- ppm
- retardation factor (R_f)
- retention time (R_t)
- solute
- solvent
- solvent front
- stationary phase
- thin-layer chromatography (TLC)
- vaporise

Key formulas

Retardation factor	$R_f = \frac{\text{distance solute moves from origin}}{\text{distance solvent moves from origin}}$

Revision questions

Multiple choice

1 The measurement used to calculate the concentration of a substance from a series of standards in gas chromatography is called:
- **A** retention time
- **B** peak area
- **C** peak height
- **D** retardation factor.

2 Column chromatography was performed to determine the pigments within a plant sample. The chromatogram shown in Figure 1 was analysed. What conclusions could be made about the blue pigment?
- **A** It has a high R_t and a high affinity to the stationary phase.
- **B** It has a low R_t and a high affinity to the stationary phase.
- **C** It has a high R_t and a high affinity to the mobile phase.
- **D** It has a low R_t and a high affinity to the mobile phase.

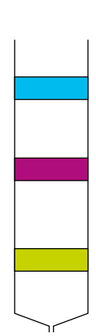

FIGURE 1 A chromatogram of plant pigments.

3 Which of the following would result in the highest R_f value?

	Mobile phase	Stationary phase	Substance
A	polar	non-polar	polar
B	non-polar	polar	polar
C	polar	non-polar	non-polar
D	non-polar	polar	polar

4 In HPLC, R_t can be decreased by:
- **A** lowering the temperature
- **B** decreasing the pressure
- **C** increasing the flow rate
- **D** decreasing the flow rate.

5 Which chromatographic techniques are qualitative as well as quantitative?
- **A** Paper chromatography, TLC, gas chromatography and HPLC
- **B** TLC and gas chromatography
- **C** Gas chromatography and HPLC
- **D** HPLC only

Short answer

Describe and explain

6 Explain how chromatography separates substances from a mixture. Use the terms 'mobile phase', 'stationary phase', 'affinity', 'adsorb' and 'desorb'.

7 Describe the difference between a qualitative and quantitative analysis in chromatography. Use examples in your answer.

8 Explain why it is necessary to prepare a set of standards when performing a quantitative analysis in chromatography.

Apply, analyse and interpret

Questions 9–12 are based on the following information.

Two disinfectant sprays were analysed by HPLC to determine whether the ethanol values specified on the labels were accurate.

The data in Table 1 was obtained from a series of standards of ethanol solution.

TABLE 1 Peak areas of ethanol standards

Standard	Concentration of ethanol (% per100 mL)	Peak area (cm^2)
0	0	0
1	3	2
2	6	4
3	9	6
4	12	8
5	15	10

The two disinfectant samples were run through the HPLC instrument and the data in Table 2 was obtained.

TABLE 2 Peak areas of ethanol in two disinfectants

Sample	Label ethanol content (% per 100 mL)	Peak area (cm^2)
Disinfectant 1	1–3	3
Disinfectant 2	6–8	5

9 Draw a calibration curve for the ethanol standards.

10 Determine the concentration of ethanol in the two samples.

11 Infer the value of the product labels based on the calculations of concentration from the previous question.

12 The standards that were used varied in concentration between 0 and 15% per 100 mL. Consider why this range was selected.

Investigate, evaluate and communicate

13 An HPLC analysis was performed to determine the R_t of a polar substance by using a polar mobile phase and non-polar stationary phase.

a Determine the effects of decreasing the polarity of the mobile phase on the R_t of the substance. Justify your answer.

b Construct a chromatogram that demonstrates this effect.

14 A student wanted to determine the concentration of methane in natural gas by gas chromatography. After running a set of standards with a flow rate of 1 mL min^{-1}, he determined that this was too slow and increased the flow rate for the samples to 5 mL min^{-1}.

a Can the student generate a calibration curve?

b Is it possible to determine the concentration of methane?

c Is it possible to determine the identity of methane?

d Is the student's data valid?

15 Synthesise the chromatographic techniques into a table such as the one below.

TABLE 3 Summary of chromatographic techniques

Chromatographic technique	Mobile phase	Stationary phase	How the data is collected and analysed	What types of analyses this is used for	Qualitative or quantitative?
Paper chromatography					
TLC					
Gas chromatography					
HPLC					

More questions to come

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