28 CHROMATOGRAPHY

Student Learning Outcomes

[C-12-E-29 to C-12-E-33]

- Describe the terms stationary phase, mobile phase, R_f value, baseline, and solvent front.
- Explain the principles and applications of thin-layer chromatography in forensic chemistry and analysis of unknown materials.
- Interpret R_f values and retention times in chromatograms to determine the composition of a mixture.
- Explain the importance of selecting the appropriate stationary and mobile phases in chromatography and their impact on the separation of compounds.
- Describe the use of mass spectrometry in combination with chromatography for identifying and quantifying small amounts of unknown materials in forensic analysis.

28.1 INTRODUCTION

Separating one or two components from a simple mixture needs simple techniques such as filtration, distillation, and solvent extraction. However, complex mixtures require advanced techniques. Chromatography is a versatile and important analytical technique that allows chemists to separate the individual components of a complex mixture based on their varying affinities for two different phases. Complex mixtures, such as dyes in blooming flowers, pollutants in a water body, or medicinal extracts from the plant leaves, can be separated using chromatography. Use of chromatography allows for the identification, purification, and quantification of substances with high precision, making chromatography an efficient modern chemical analytical tool in medicine, forensic, environmental science, etc.

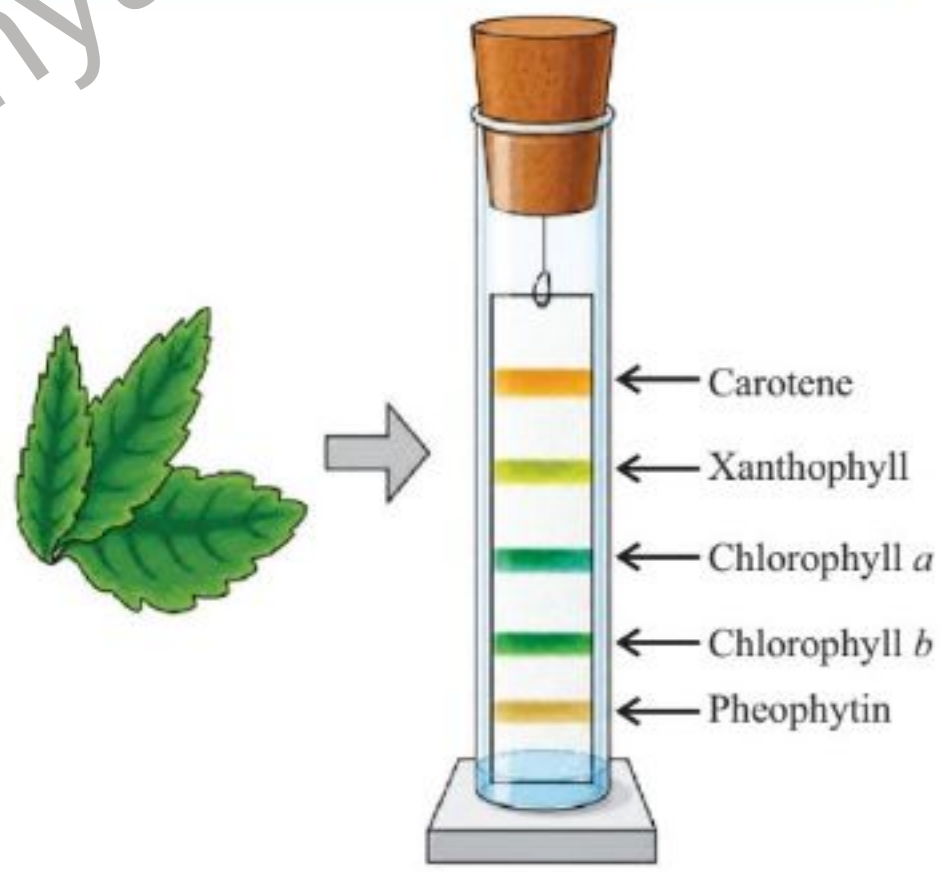


Figure 28.1 The complex mixture of pigments in leave can be efficiently separated using chromatography

Adsorption is a surface phenomenon in which particles accumulate only on the outer surface. For example, activated charcoal adsorbs gases and impurities onto its surface, which is why it's used in filters and purification systems. Among many types of chromatography, the three important types are:

1. Paper chromatography (PC)
2. Thin layer chromatography (TLC)
3. Gas chromatography (GC)

28.2 PAPER CHROMATOGRAPHY

The technique of using a solvent to separate a mixture into its components is called paper chromatography. The separating principle depends on relative solubility of the components. The piece of paper used in chromatography contains water combined with cellulose of the paper. A small spot of the sample is put on the base line near the bottom of the paper by capillary tube. The paper is dipped in a suitable solvent which will soak up the paper. The solvent level must be below the spot of the sample. The solutes present in the sample dissolve in the solvent to different extents. Some are more soluble in the solvent (mobile phase) moving up the paper. Others dissolve better in the water trapped in the paper (stationary phase); therefore, they do not travel very far up the paper. This difference in the solubility allows the different components in the sample to be separated.

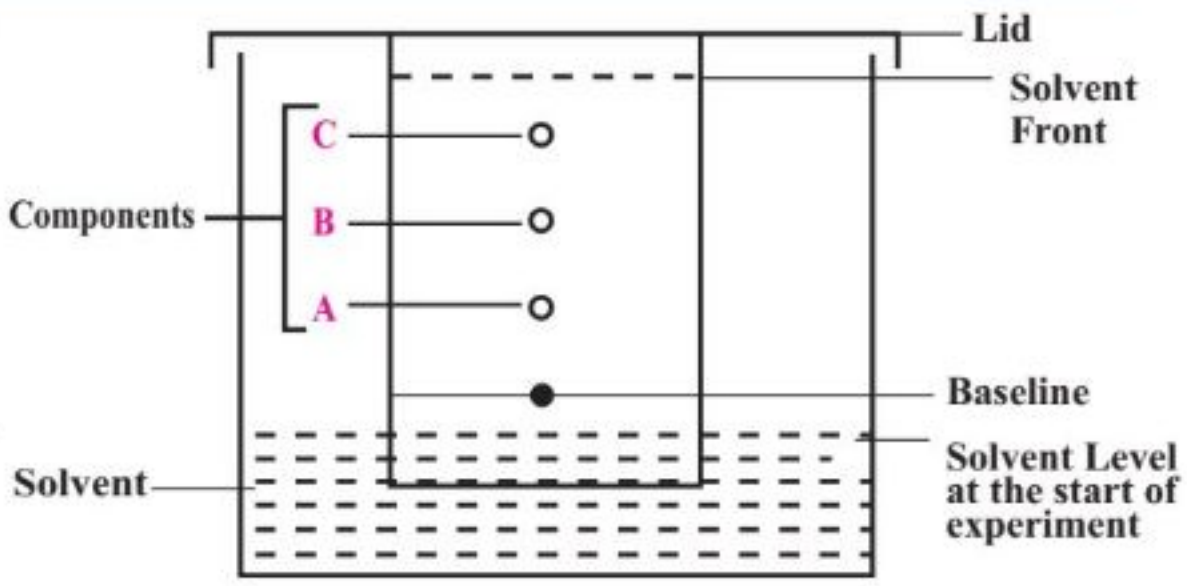


Figure 28.2 Paper Chromatography

Chromatography is used to separate both coloured and colourless substances. Colourless substances can be visualised by spraying them with a locating agent which give colour to them.

The R_f value (Retardation factor or Retention factor) of a substance is calculated by dividing the distance traveled by the component with the distance travelled by the solvent.

$$R_f = \frac{\text{Distance traveled by the substance}}{\text{Distance traveled by the solvent}}$$

R_f has no unit because it is ratio. The R_f value of a substance does not change under same conditions (same solvent and same temperature). One advantage of using chromatography is that it consumes very small quantity of the mixture.

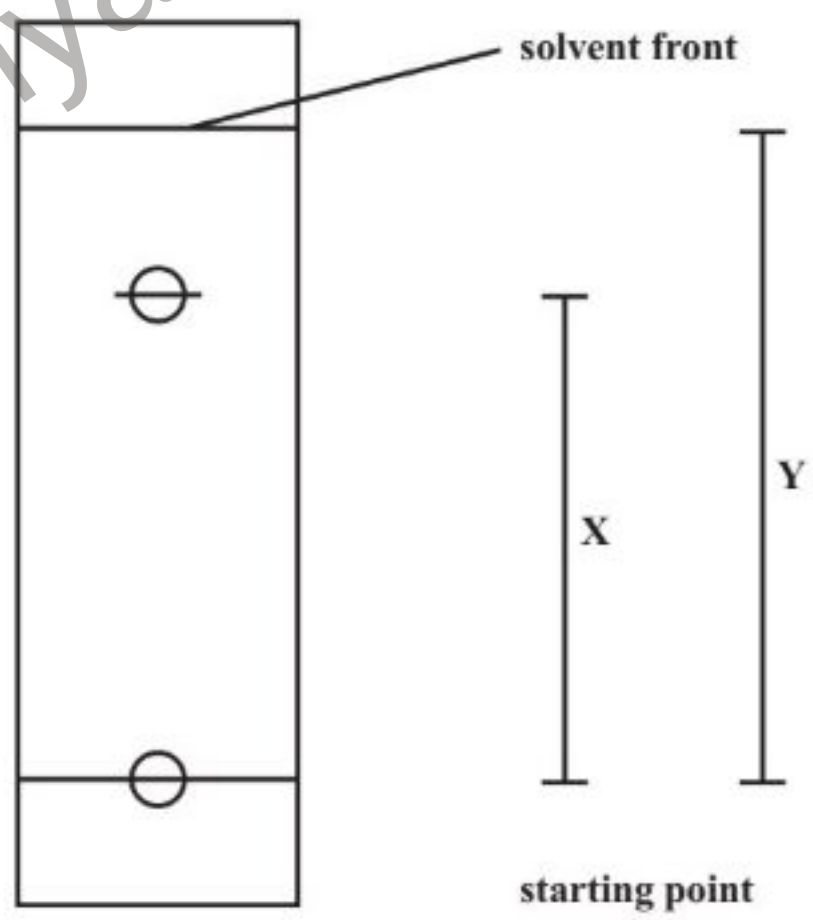


Figure 28.3 Measuring distance of spot and solvent front

28.3 THIN-LAYER CHROMATOGRAPHY

In thin layer chromatography (TLC), alumina (Al_2O_3) or silica (SiO_2) is mixed in water to make slurry which is then spread evenly onto a glass slide. This layer is dried in an oven to make a white solid layer (stationary phase) on the glass slide. TLC is faster than paper chromatography.

A straight baseline is drawn by pencil and ruler near the bottom of the glass slide. A spot of sample

is put on the baseline by the capillary tube. More than one samples can be spotted at different places on the same glass slide. The solvent is taken in a chromatography tank. The glass slide is dipped in the solvent (mobile phase) in such a way that baseline is above solvent level.

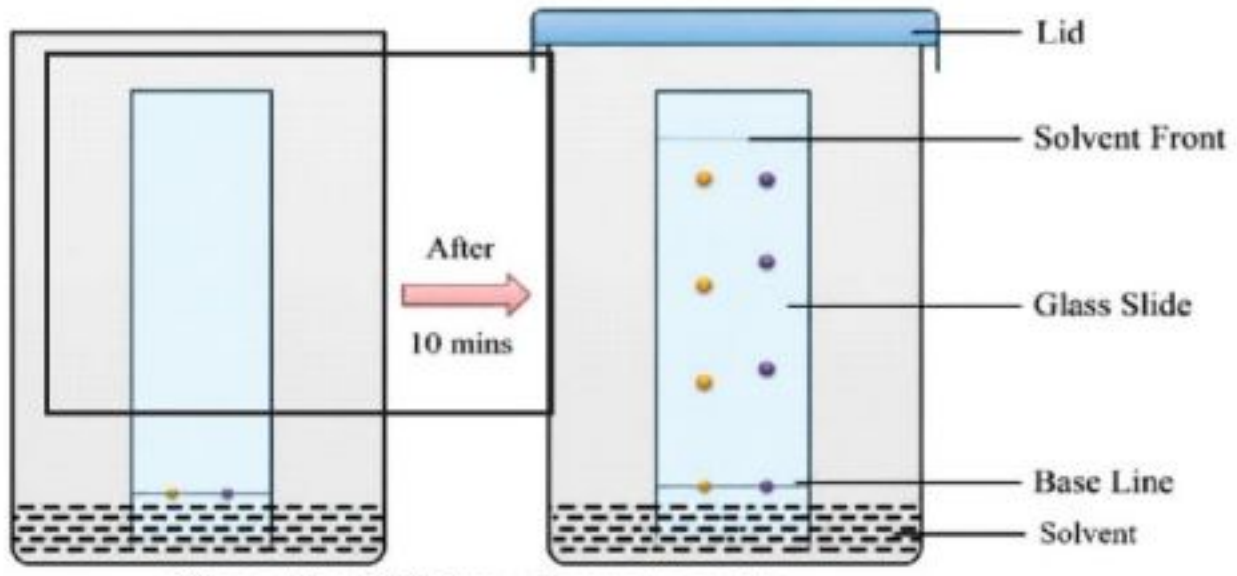


Figure 28.4 Thin layer chromatography

The components present in the sample dissolve in the solvent and adsorb onto aluminium oxide differently. Some are more soluble in the mobile phase while some are more adsorbed onto the solid stationary phase. Polar substances have greater attraction for polar solid stationary phase and are adsorbed more strongly. Therefore, they travel more slowly up the stationary phase. On the other hand, non-polar substances are more soluble in the non-polar solvent, and they move more up the slide. This difference allows the substances in the sample to separate. After the solvent has moved up to a certain height, the glass slide is removed from the chromatographic chamber. Solvent front, the highest point reached by the solvent, is marked. The glass slide is dried by dryer. R_f values of different coloured spots are calculated.

By comparing these R_f values with standard values, the components present in the sample are identified. Sometime the components are colourless, locating agent is sprayed to visualise them. Ninhydrin spray give purple colour to colourless amino acids. Some colourless substances glow when irradiated with ultra-violet light while some turn brown with iodine vapours.

Applications:

i) TLC identify unknown compounds by comparing their R_f values with standard known values.

ii) The purity of a substance can be checked by TLC.

iii) The progress of reaction whether reactants are consumed or products are made, can be checked by TLC.

iv) In pharmaceutical and food industry, purity of drugs and presence of preservatives, colourants, sweeteners in food can be checked by TLC.

v) TCL can also detect pollutants, pesticides, toxic substances in soil, water, food and air.

vi) TLC is useful in forensic science where it is used to identify drugs, post blast debris, blood, sweat, semen at crime scene.

Limitations:

i) TLC is mainly a qualitative technique and can only give an idea about the amount of a substance. So, TLC is not accurate and precise quantitative technique.

ii) TLC cannot clearly separate compounds having close R_f values. Hence resolution of spots is not always high and sharp.

iii) In TLC, only small amounts of sample can be applied and analysed. It is not suitable for large-scale separation.

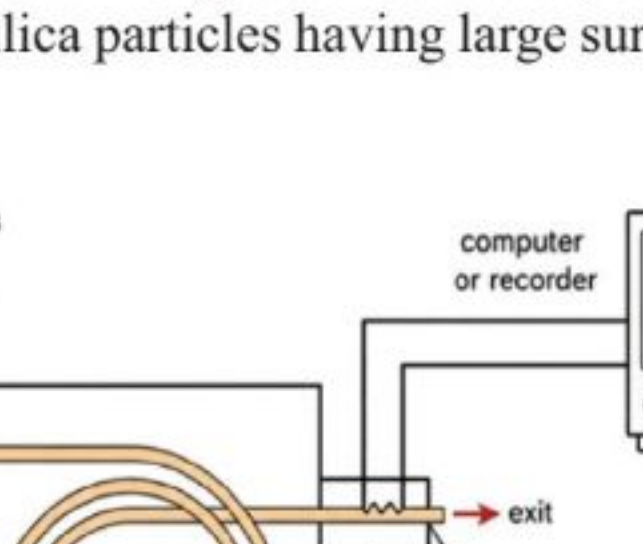
iv) This technique is sensitive to humidity, temperature and cleanliness of working environment which reduces reproducibility.

Quick Check 28.1

a) A mixture contains butanone and pentane. How can they be separated by using thin layer chromatography using aluminium oxide as stationary phase and benzene as solvent?

b) A mixture of two substances X and Y is analysed by thin-layer chromatography. The R_f value of substance X is larger than that of substance Y. Suggest why substance X has a larger R_f value.

c) A separate thin layer chromatography experiment was done using the hair dyes A, B and C. The chromatogram obtained is shown. State three conclusions about the hair dyes A, B and C which vcan be deduced from the chromatogram.



28.4 GAS CHROMATOGRAPHY

Gas chromatography (GC) is used to separate gases, volatile liquids and solids (in gaseous form). In GC, the stationary phase is a non-volatile, long chain, non-polar hydrocarbon liquid having high boiling point bonded to small particles of silica (SiO_2) or alumina (Al_2O_3). In GC, the mobile phase is an inert gas (carrier gas) such as helium or nitrogen. The sample is injected into injector port, where sample is quickly vapourised by an oven. The carrier gas carries the vapours of the sample through a long-coiled column packed with tiny silica particles having large surface area over which separation occurs.

The non-polar substances in the mixture have greater interaction with non-polar liquid on the solid particles of stationary phase. Therefore, these non-polar substances are carried through the column more slowly. Thus, substances that interact more strongly move slower while other move faster, resulting in separation. As the separated substances exit the column, they pass through a detector which identify and record them as peaks on a graph called a chromatogram. Detector

Figure 28.6 Gas Chromatography

Figure 28.5 Gas chromatography

also records retention time of each component. The time taken by a substance to pass through the column is retention time.

Gas chromatography has high sensitivity, good resolution, fast analysis and accurate quantification. Mixture of gases can easily be separated by GC at room temperature. GC is also a quantitative technique. The peaks obtained are first identified and then area of each peak is measured which will give the proportion of each component in the mixture. The peaks are similar to triangles, therefore, the area of peak can be calculated by the following formula.

$$\text{Area of a peak} = \frac{1}{2} \times \text{base} \times \text{height}$$

The percentage of an ester A in the mixture of three esters A, B and C is given by,

$$\% \text{ of A} = \frac{\text{peak area of A}}{\text{sum of peak areas of A, B and C}} \times 100$$

If the peaks are very narrow or have similar base widths then peak height is used instead of area to estimate the proportion of components in a mixture. GC is used to check the presence of steroids in competing athletes. This is also used to test the components of fuel used in car racing.

Applications:

i) In the industrial sector, GC is vital for quality control and environmental protection. For example, Petrochemical plants use it to analyse the composition of fuels, and for detecting trace amounts of organic pollutants in environmental samples.

ii) Forensic laboratories rely heavily on gas chromatography to provide evidence in legal investigations. It is frequently used to perform blood alcohol content (BAC) tests. Additionally, GC is used to detect illegal drugs, poisons, or performance enhancing substances in biological samples.

iii) The food and beverage industry utilises GC to ensure the consistency and safety of products. It can detect spoilage by identifying metabolic byproducts of bacteria or fungi before they are visible to the eye. Furthermore, GC is used to analyse the complex aromatic profiles of perfumes, essential oils, and food flavourings.

iv) In clinical settings, gas chromatography is used to diagnose metabolic disorders by analysing specific compounds in a patient's breath or urine. It also plays a role in pharmaceutical research, where it is used to test the purity of newly synthesized drugs, ensuring that no harmful residual solvents or impurities remain in the final medication before it reaches the patient.

MORE INFO

Interesting Information

Chromatography is important in forensic science. A man in China, namely Nian Bin, was accused of murdering two neighbouring children by mixing rat poison (a fluoroacetate) in their food in 2005. However, after 8 years, in 2013, he was proven innocent and released. The food sample was tested using gas chromatography, which proved no mixing of the poison.

Limitations:

i) GC works only for volatile (easily vaporised) substances.
ii) Samples must be thermally stable. Compounds that decompose at high temperatures cannot be used.
iii) Large biomolecules like proteins and polymers cannot be analysed by GC because they do not vaporise easily.
iv) Instruments and maintenance costs for GC are high.

Quick Check 28.2

An impure sample of lactic acid, $CH_3CH(OH)COOH$ contains butanone as the only contamination. The mixture is analysed using gas chromatography. Butanone is found to have a longer retention time than lactic acid.

i) Suggest suitable substances that could be used as the mobile and stationary phases.
ii) Describe how the percentage composition of the mixture can be determined from the gas chromatogram.

28.5 Comparison of three types of chromatography

Feature	Paper Chromatography	TLC (Thin-Layer)	GC (Gas Chromatography)
Principle	Partition	Adsorption + solubility	Partition (gas-liquid)
Mobile Phase	Liquid	Liquid	Gas
Stationary Phase	Water in paper	Silica/alumina	Non-volatile liquid
Speed	Slow	Faster	Very fast

Exercise

Q1. MULTIPLE CHOICE QUESTIONS :

I) Why must the solvent level be below the spot of the dye?

a) To increase temperature
b) To prevent the dye from dissolving directly into the solvent
c) To speed up chromatography
d) To increase adsorption

II) Why do some components of a dye travel further up the paper than others?

a) They are more soluble in the water trap in paper (stationary phase)
b) They are more soluble in the solvent (mobile phase)
c) They react with the paper
d) They have higher density

III) A substance has a very low R_f value. What does this indicate?

a) It is insoluble in the solvent
b) It is highly soluble in the solvent
c) It strongly sticks to the water inside paper
d) It has a high density

IV) Which statement best explains adsorption?

a) Substance dissolves completely in another
b) Substance sticks only to the surface of another material
c) Substance changes its state
d) Substance reacts chemically

V) A polar compound in TLC will usually:

a) Travel farther up the plate
b) Stay near the baseline
c) Evaporate quickly
d) Dissolve completely

VI) Why is TLC considered faster than paper chromatography?

a) It uses more solvent
b) It has a solid stationary phase that allows quicker separation
c) It requires higher temperature
d) It uses colored substances only

VII) What is the role of the carrier gas in gas chromatography?

a) To react with the sample
b) To act as stationary phase
c) To carry vaporized sample through the column
d) To detect the substances

VIII) Why must the stationary phase in GC have a high boiling point?

a) To evaporate quickly
b) To increase pressure
c) To remain in liquid form during the process
c) To react with gases

IX) What does the area under a peak in a chromatogram indicate?

a) Amount (proportion) of the substance
b) Retention time
c) Temperature of column
d) Color of compound

X) If two peaks have similar base width but different heights, how can composition be estimated?

a) By color
b) By retention time
c) By pressure
d) By peak height

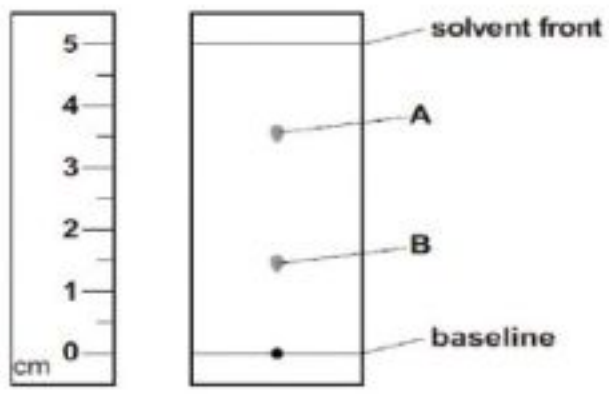
Q2. SHORT ANSWER QUESTION:

a) What is the principle of paper chromatography?
b) Why is pencil used instead of ink in chromatography to mark the basline?
c) State two advantages of TLC over paper chromatography.
d) Why do polar substances move slowly in TLC?
e) Why is TLC not suitable for large-scale separation?
f) What is the function of injector port in GC?
g) Why must samples be volatile in GC?
h) State two limitations of gas chromatography.
i) What information does a chromatogram provide?

j) What is adsorption? Give one example.

Q3. CONSTRUCTED RESPONSE QUESTIONS

a) A mixture of amino acids is analysed using thin layer chromatography. The chromatogram obtained is shown. The table shows some R_f values for different amino acids in the same solvent.



amino acid	*Rf* value
alanine	0.40
glutamic acid	0.29
leucine	0.71
valine	0.61

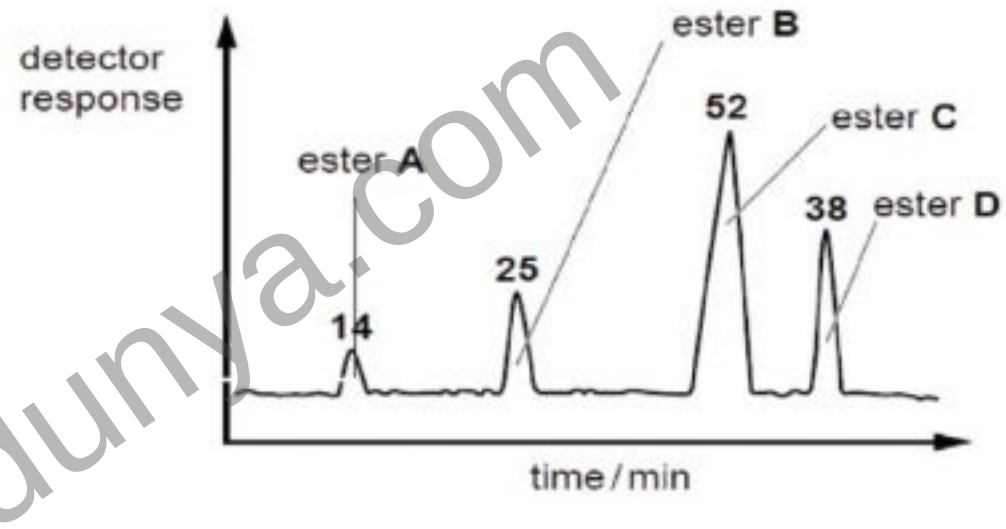
I) Use the chromatogram and the R_f values to deduce the amino acid responsible for spot A and spot B.

ii) A second chromatogram of the same mixture is taken using a more polar solvent. Predict the effect on the R_f values of the amino acids. Explain your reasoning.

3. Gas chromatography involves a stationary phase and a mobile phase.

a) Name a suitable substance that could be used for each phase.

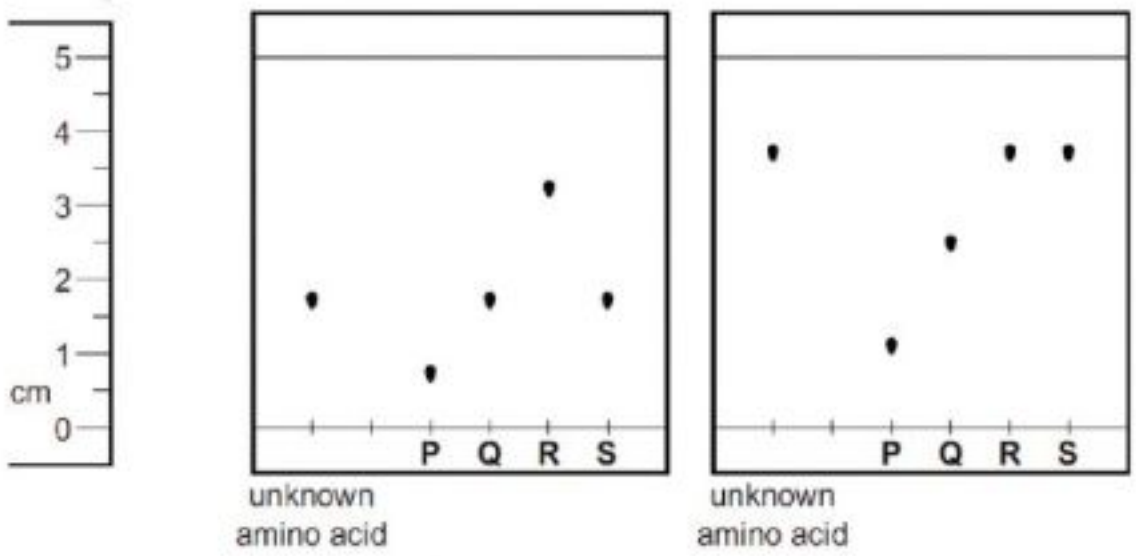
b) A mixture of three organic compounds is separated by gas chromatography. The chromatogram obtained is shown below.



I) Explain the meaning of the term retention time.

ii) Calculate the % of N in the mixture. Show your working.

c) An unknown amino acid is analysed by thin-layer chromatography. Two chromatographs of the unknown amino acid and four reference amino acids, P, Q, R and S, are obtained using two different solvents. Identify the unknown amino acid. Justify your answer.



DESCRIPTIVE QUESTIONS

Q4. Describe the principle and working of paper chromatography.

Q5. Explain the principle and working process of thin layer chromatography (TLC) in detail.

Q6. Describe the main components of gas chromatography.