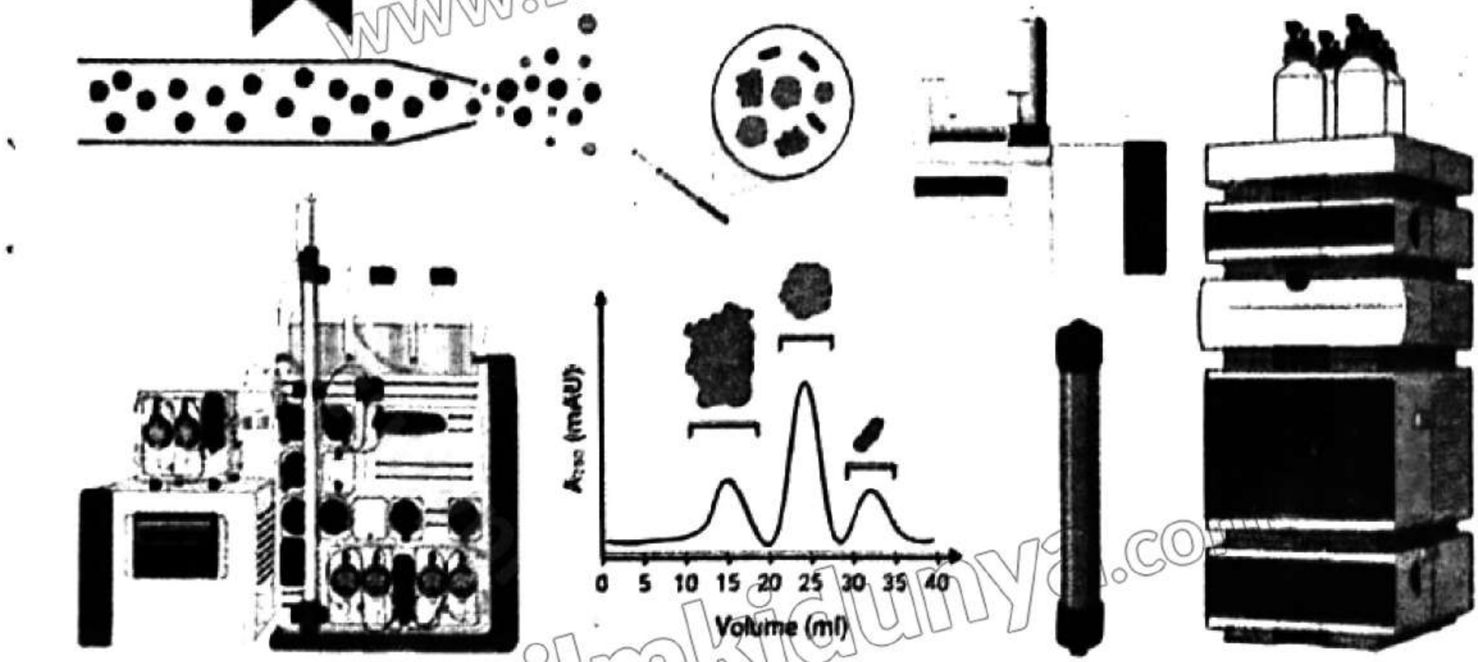
UNIT 19



CHROMATOGRAPHY

Student Learning Outcomes (SLOs)

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

Chromatography is a fascinating lab technique that is useful for separating mixtures. It plays a vital role in various fields for example helping scientists to identify the components of medicines, assess the purity of water, and analyze the nutritional content of foods. Its name comes from Greek words meaning "color" and "to write," highlighting its ability to visually represent the different parts of a mixture based on their colors.

It has several significant applications in many pharmaceutical sectors, food and chemical industries. Chromatography is used to check the purity of compound. Environmental testing laboratories are using these techniques of Chromatography. It is also used in beverage, forensic and drug testing. One of the key advantages of chromatography is its ability to separate complex mixtures into their individual components, providing valuable information about composition and purity of compounds.

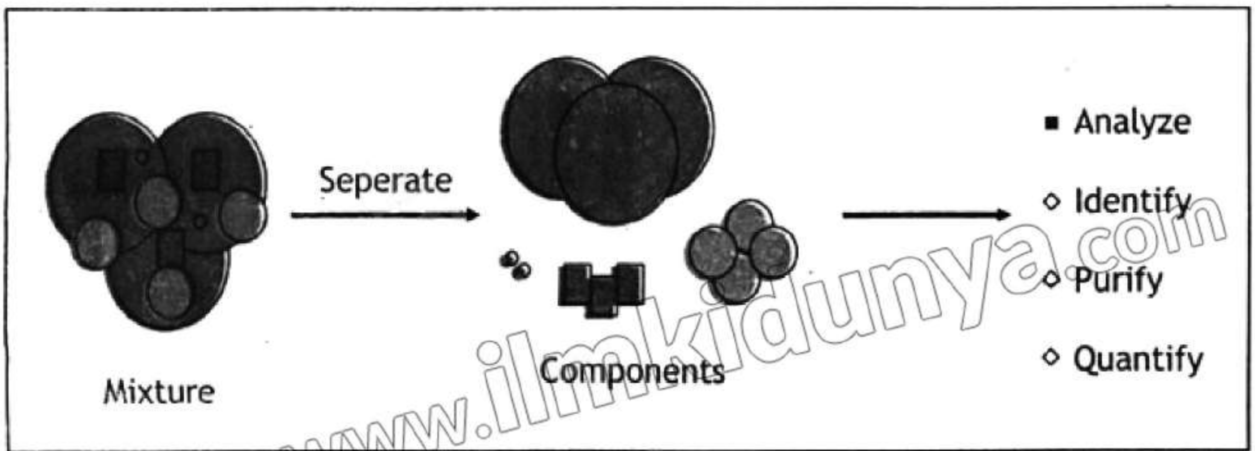


Fig 19.1: Process of chromatography

19.1 Main Components of Chromatography

Chromatography involves two phases: one that is stationary phase (usually a solid like glass or silica) and other one is mobile phase (usually a liquid or gas).

1. Stationary phase: The stationary phase doesn't move, while the mobile phase moves over it. The stationary phase is often packed into a tube while the mobile phase flows through it for example in column chromatography the stationary phase is packed in a column and mobile phase is run through the column. Stationary phase can be in solid or liquid phase coated on surface of solids.

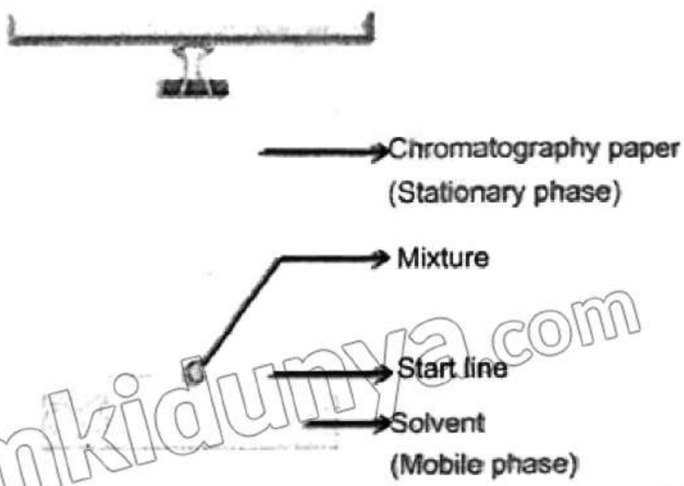


Fig:19.2 Stationary and mobile phase

2. Mobile phase: In chromatography, the mobile phase is usually a liquid or gas that flows over the stationary phase. Liquid chromatography uses a liquid mobile phase, and gas chromatography uses a gas mobile phase. In Column chromatography, the mobile phase (either liquid or gas) passes through a column separating the mixture's components by sticking to the stationary phase at different rates.

3. R_f (retardation factor) value: Rf value of a compound is the ratio of the distance travelled by the solute to the distance travelled by the solvent.

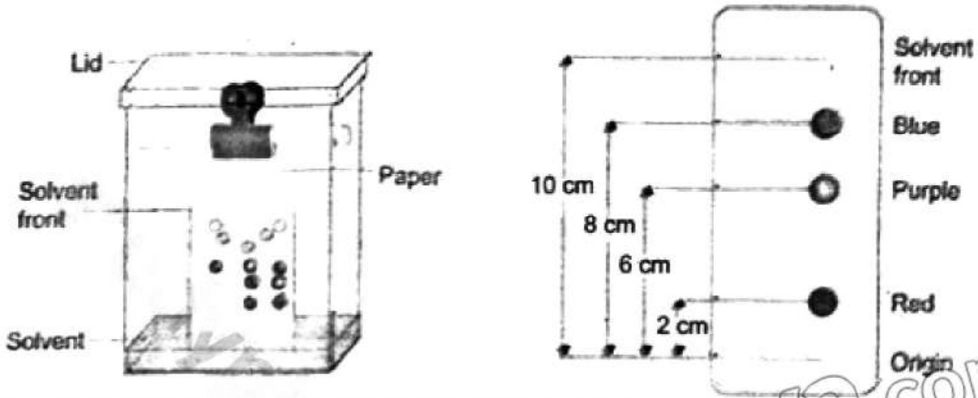


Fig :19.3 R_f calculations

In chromatographic analysis retention factors (R_f) is used to tell that how far a compound travels compared to the solvent front, providing valuable information for compound identification. It is calculated as the ratio of the distance travelled by the compound to the distance travelled by the solvent front from the baseline, where the sample was initially applied.

$$R_f \text{ Value} = \frac{\text{Distance from Baseline travelled by Solute(compound)}}{\text{Distance from base line travelled by solvent(Solvent front)}}$$

4: Solvent front: The level at which solvent reaches as it moves up the paper is called the solvent front. There is a dynamic equilibrium between the mobile and stationary phases as the components constantly move between the two phases.

5. The baseline: The baseline is the starting line from which the movement of components is measured. It is typically represented as a straight line on the chromatogram and is used as reference point and spots are placed at this level.

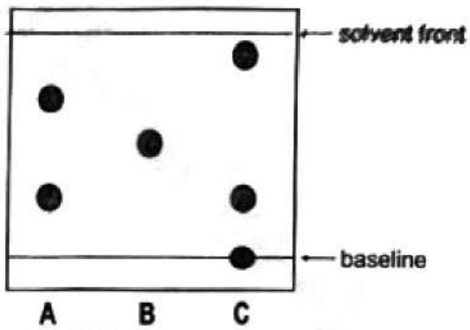


Fig 19.4 Paper Chromatogram

Do you Know

Chromatography is used in biochemical research for the separation and identification of chemical compounds of biological origin. In petroleum industry the technique is employed to analyze complex mixture of hydrocarbons.

19.2 Thin Layer Chromatography

Thin-layer chromatography (TLC) separates substances based on how they interact with different surfaces. In TLC, substances move across a thin layer on a plate. Some substances stick more to this layer (the stationary phase) and move slowly, while others move faster. This separates the mixture into different parts, which appear as spots on the plate.

In the process of thin-layer chromatography (TLC), the mixture of substances are separated into its components with the help of a glass plate coated with a very thin layer of adsorbent, such as silica gel and alumina, as shown in the figure below.

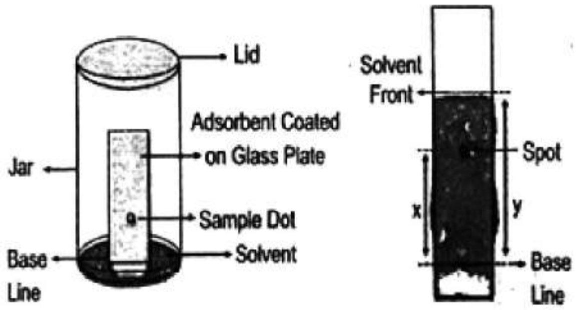


Fig 19.5: Thin Layer Chromatography

The solution of the mixture to be separated is applied as a small spot at a distance of at least 2 cm. The plate is then placed in a closed jar containing a liquid termed as an eluant, which then rises up the plate carrying different components of the mixture to different heights.

TLC is one of the fastest, least expensive, simplest and easiest chromatography technique. Thin Layer Chromatography plates are pre-made plates that are chemically inert and stable. They have a thin layer of stationary phase applied to their surface. This layer is fine and evenly thin.

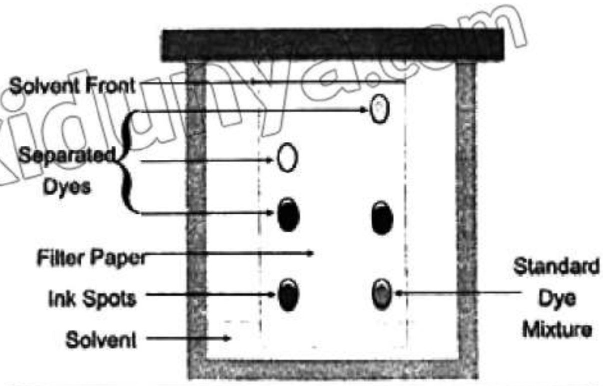


Fig 19.6 Chromatography Chamber

The plates are developed in a Chamber. It maintains a stable environment inside to help spots develop properly. It also prevents solvent evaporation and keeps the process free from dust.Mobile Phase in Thin Layer Chromatography is a solvent mixture or a single solvent. It needs to be free from particles. The purer the mobile phase, the better the spots will develop.

19.2.1 Working of thin Layer Chromatography

1. The stationary phase on the plate is coated with silica gel or Aluminum oxide and dried.
2. Use a pencil to make small marks at the bottom of the plate. Apply sample solutions to these marks by using capillary tube.
3. Pour the mobile phase in TLC chamber and add moistened filter paper to keep the humidity constant.
4. Put the plate in the chamber with the sample and close it with a lid.
5. Let the plate develop, making sure the sample spots stay above the mobile phase level without dipping into the solvent.
6. Once spots develop, remove the plates and let them dry. We can check the sample spots under a UV light.

Group Activity:

Design an experiment to study the different component of following mixtures using thin layer chromatography.

(i) Colors of markers.
(ii) Pigments present in petals of hibiscus flower
(iii) Component of ink

Hint:

Teacher will provide

(i) three different brands of black water-based markers.
(ii) TLC plates
(iii) hibiscus flowers petals along with some small amount of alcohol and mortal and pestle
(iv) Sample of different kinds of inks.

19.2.2 Applications of thin Layer Chromatography (TLC)

1. Testing various medicines like sedatives, local anesthetics, and more.
2. Useful in biochemical analysis, separating substances obtained from food.
3. Identifying natural products such as essential oils and alkaloids.
4. Purifying samples and comparing them with authentic ones.
5. In the food industry, for separating and identifying colors, sweeteners, and preservatives.
6. Used in the cosmetic industry.
7. Useful in Organic synthesis.

19.2.3 Limitations of thin layer chromatography

1.Separation length is shorter compared to other chromatography methods.

2.Being an open system, factors like humidity and temperature can influence the final results.

3.This method is suitable for detecting very small amounts of substances.

19.2.4 Application of thin layer Chromatography in Forensic Chemistry

The application of TLC in forensic chemistry contributes significantly to the investigation and resolution of criminal cases by providing rapid and reliable separation and analysis of diverse forensic samples. Thin layer chromatography (TLC) finds several applications in forensic chemistry due to its ability to separate and analyze compounds within complex mixtures. Here are some key applications of TLC in forensic chemistry.

1. **Drug Analysis:** TLC is extensively used in the analysis of illicit (forbidden by law) drugs. Forensic chemists can separate and identify various drugs present in samples, such as cocaine and *heroin*. By comparing the separated compounds with known standards, scientists can determine the composition and purity of the drugs.

2. **Poison Report:** In cases of suspected poisoning, TLC can be employed to analyze biological samples (e.g., blood, urine, tissues) for the presence of toxic compounds. This helps forensic scientists identify the poison involved.

3. ***Trace Evidence findings:*** TLC is valuable for analyzing trace evidence found in fibers, paints, and dyes. By separating and comparing the components of these materials with known standards or reference samples, forensic experts can link them to specific sources, aiding in criminal investigations.

4. **Gunshot Residue Analysis:** TLC can be used to analyze gunshot residue (GSR) collected from suspects or crime scenes.

5. **Explosives Inspection:** Thin Layer Chromatography (TLC) is used to detect and analyze explosive residues. By separating and identifying components like nitroaromatics or nitrates, forensic chemists can determine if explosives are present in samples from crime scenes or suspected bomb-making facilities.

6. **Document Analysis:** Thin Layer Chromatography (TLC) helps to analyze ink and paper samples in questioned document cases. By separating the components of inks or paper coatings, forensic experts can compare questioned documents with known samples helping in document authentication or detecting forgeries.

7. **Scientific Screening:** Thin Layer Chromatography (TLC) is a preliminary screening tool in toxicology. It quickly identifies and separates drugs in biological samples, helping forensic scientists decide which samples need further analysis with more sensitive techniques like GC-MS or LC-MS.

In forensic chromatography labs, various techniques are used, each serving different purposes. These include gas chromatography, high-performance liquid chromatography (HPLC).

19.3 Interpretation of R_f Value

Retention factor (R_f) values in chromatography show how much a solute likes the stationary phase or the mobile phase. This helps to understand its properties, such as how polar it is, its relative size, and how well it dissolves.

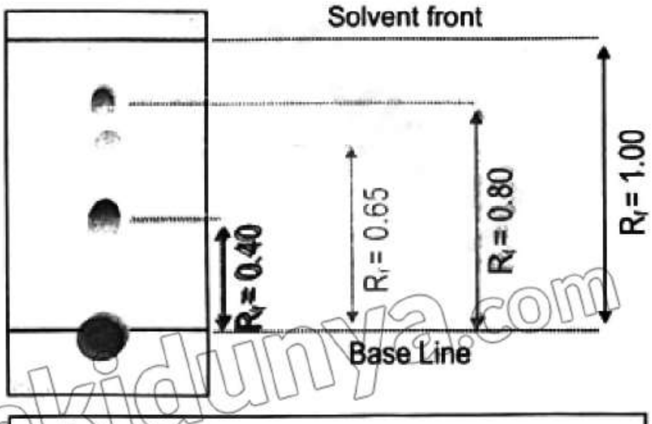


Fig 19.7 R_f Value

Consistent R_f values allow for the identification of unknown substances by comparing them to known substances. Different R_f values indicate different compounds, while similar values suggest possible identity. Slight variations can occur due to interactions and concentration differences. Overall, R_f values are essential for analyzing properties and comparing substances in chromatography.

19.3.1 Factors Affecting R_f Values

On the chromatography paper, a prepared sample solution containing component(A+B) is applied and processed through a mobile phase. Because of their different affinities with the mobile phase, analytes (A) and (B) are separated. The analytes, solvent front, and the point where the mixture (A+B) was administered are all measured relative to each other.

1. Solvent Impacts Retention Factors

As solvent transports the chemical along the plate, the solvent used has a significant impact on the chemical's retention factor value. Since R_f value is the ratio of distance travelled by solute and solvent, therefore solvent is the most important factor which affect the R_f value. It is observed that R_f value of compound is higher in polar solvent as compared to non-polar solvent. Sometimes mixture of solvents is used for TLC to adjust the R_f value.

2. Solute (Sample)

Solute or sample are also responsible for R_f value. The compounds which contain polar groups such as hydroxyl (OH) or amine (NH_2) are able to bind with silica of TLC plate. Therefore, it travels slower as compared to solvent. Therefore, R_f value to the polar compound is lower. Whereas non-polar compounds bind to the silica with a lesser extent. Hence solvent and solute travel faster towards the top end. Thus, R_f value of non-polar compounds is higher than polar compounds.

3. Temperature

At higher temperatures solubility of compounds is more therefore temperature affects the R_f values of a compound.

4. Thickness of silica layer

Silica of the TLC plate binds with solute/compound, thus thicker the silica layer stronger the binding and this results in lowering the R_f value.

19.3.2 Retention time

Retention time is used in column chromatographic technique. Retention time is the time taken for a sample molecule to travel through the column, from the time it is inserted into the machine to the time it is detected. Molecules in the gaseous mixture travel at different rates, therefore giving rise to different retention times.

a) Longer retention times are associated with:

Non-polar components in the mixture

They are more attracted to the non-polar liquid in the stationary phase

So non-polar molecules travel slower through the column

b) Shorter retention times are associated with:

Polar components in the mixture that prefer to interact with the carrier gas.

They are less attracted to the non-polar liquid in the stationary phase.

So polar molecules travel faster through the column.

These molecules may have lower boiling points and, therefore, are vaporized more readily.

19.4 Selection of Mobile and Stationary Phase

19.4.1 Selection of stationary phase

In general, an adsorbent is used as a stationary phase during the process of chromatography. It should have the following characteristics:

The stationary phase functions as an adsorbent and possesses the following traits

1. High and selective adsorption capacity
2. Finely divided to maximize surface area for adsorption
3. High mechanical stability to minimize dust formation
4. Chemically inert towards sample and eluting solvents
5. High purity
6. It should be easily available

The stationary phase is selected in such a way that the components of the sample have different solubilities in the phase. Hence, different components have different rates of movement through the stationary phase and as a result, can be separated from each other.

The second step in choosing the stationary and mobile phases is to determine the desired separation that you want to achieve.

If you want to separate a mixture of closely related compounds with high resolution, you can use a stationary phase with small particles and a mobile phase with low viscosity and high flow rate. This way, the components will have less diffusion and more interaction with the stationary phase, resulting in sharper peaks and better separation.

19.4.2 Selection of Mobile Phase in Chromatography

When choosing the right mobile phase in chromatography, factors are more important than specific factories. However, choosing solvents from reputable manufacturers with high-quality control standards is crucial for successful separations. Here are some key factors to consider.

Solvent purity

Use HPLC (High performance liquid chromatography) or MS (Mass spectrometry) to grade solvents to keep impurities low and prevent them from affecting your analysis. Buy solvents from companies with strong quality control to ensure they are pure and consistent for your experiments.

Solvent properties

Polarity: Consider the polarity of your analytes and match it to the mobile phase for optimal interaction.

Viscosity: Optimal viscosity ensures proper flow rate and peak shape.

Chemical reactivity: Choose solvents that are stable and won't degrade your analytes or the stationary phase.

UV transparency: If using UV detection, the solvent shouldn't absorb UV light at detection wavelength.

pH: For methods involving charged solute pH control is crucial.

19.5 Use of Mass Spectrometry in Forensic Analysis

Mass spectrometry is an important analytical tool used in chemistry, biochemistry, pharmacy, medicine, and many related fields of science. It helps to analyze and investigate single cells and objects from outer space. Mass spectrometry is crucial for identifying the structure of unknown substances, analyzing environmental and forensic samples, and ensuring the quality control of drugs, foods, and polymers.

Mass spectrometry is a scientific method used in forensic science to analyze tiny bits of substances found at crime scenes. It helps detectives and scientists to figure out what substances are present in things like human tissue, drugs, or chemicals.

> Mass spectrometers are instrumental in detecting and identifying atmospheric pollutants. In the realm of forensics, these devices are invaluable for drug testing, toxicology studies, and even the detection of explosive residues.

19.5.1 Mass spectrometry used in forensic science

In mass spectrometry, a sample is analyzed to determine its molecular composition through the generation of a spectrum that reveals the masses of its component parts.

The sample is injected into the mass spectrometer, either directly or after passing it through a chromatography-based instrument to separate it into its constituent components. An ionization chamber turns the components into charged ions, by removing at least one electron from the sample.

This form of ionization is called electron ionization (EI), which is one of the most common methods used.

The ions can then be accelerated in a mass analyzer and separated from one another using a magnetic field, which deflects the ions to different degrees based on their masses.

In the case of EI, once the sample components are separated, ion beams then enter a detector, which generates an electric signal proportional to the number of ions hitting it. This creates a mass spectrum that shows the mass-to-charge (m/z) ratio of the individual component ions that were in the sample. This spectrum enables a forensic analyst to determine exactly which compounds the sample is composed of, using a combination of mass spectrum.

For greater resolution, forensic analysts may use more sophisticated type of spectrometry, which separates sample components based on how long it takes for them to cross a certain distance within a vacuum, rather than separating fragments based on their mass-to-charge ratios.

Overall, mass spectrometry is a powerful tool that has greatly improved forensic science. It helps scientists find important clues in complex samples and provides valuable evidence that can be used to catch criminals and ensure justice is served.

> **Bugs can help solve a crime.**
>
> It's actually called forensic entomology. While bugs can't actually solve a crime, investigators are able to look inside of an insect's (typically a maggot) stomach and is able to determine how long a body has been decomposing.

19.5.2 Applications of Mass Spectrometry

Mass spectrometry could help to determine what toxin was used or if drugs were involved, it could identify what kind of drugs they were. This information is crucial in solving crimes and providing evidence in court cases.

Mass spectrometry is an efficient method to elucidate the chemical composition of a sample or molecule. More recently, it has been used to classify biological products, in particular proteins in a number of species. Usually, mass spectrometers can be used to classify unknown substances by molecular mass measurement, to measure known compounds, and to determine the structure and chemical properties of molecules.

19.5.4 Advantages of Mass spectrometry

1. Mass spectrometry can detect substances at very low concentrations (parts per million or even parts per billion).
2. It can accurately determine the atomic composition and molecular mass of a sample.
3. Mass spectrometry can analyze a wide range of compounds, including complex mixtures.

19.5.5 Disadvantages of Mass Spectrometry:

1. Mass Spectrum is less effective for identifying compounds of hydrocarbon producing similar ions.
2. Mass spectrometry cannot separate optical and geometric isomers, which are compounds with the same molecular formula but different structures.
3. The equipment is expensive and requires specialized training to operate and interpret results.

Combining mass spectrometry with other methods, like gas chromatography, can be used for improving separation and identification capabilities.

KEY POINTS

1. Chromatography separates compounds based on their attraction to the stationary phase, which doesn't move, and the mobile phase, which carries the compounds. More polar compounds move shorter distances, resulting in a lower R_f factor. For example, glucose, being very polar, moves a shorter distance.
2. Chromatography involves combining the substance with a liquid or gaseous mobile phase, leading to the separation of different components in the sample. Each component exits the stationary phase at a specific time called retention time.
3. Chromatography is crucial in protein purification strategies and for separating, isolating, and purifying proteins from complex samples.
4. Different chromatography methods use different stationary phases; for example, paper chromatography uses water bound to cellulose fiber while thin-layer chromatography (TLC) uses a glass plate coated with silica gel.
5. R_f value is the ratio of substance distance to solvent front distance. Higher R_f values indicate lower polarity, while lower values suggest higher polarity.

6. In thin-layer chromatography (TLC), the mixture of substances are separated into its components with the help of a glass plate coated with a very thin layer of adsorbent, such as silica gel and alumina,

7. Polarity affects a chemical's attraction to other substances. More charge difference means more polarity. Increasing solvent polarity makes all mixture components move faster during chromatography.

8. Mass spectrometry, widely used in biology, chemistry, physics, clinical medicine, and space exploration, separates molecular ions based on mass and charge to determine compound molecular weight.

9. Mass spectrometry is used for both qualitative and quantitative analysis of chemical substances, helping classify sample elements and isotopes, determine molecular masses, assess sample purity, and calculate molar mass.

10. In Forensic mass spectrometry helps detectives and scientists figure out what substances are present in things like human tissue, drugs, or chemicals found at a crime.

11. During poisoning investigations, the detecting specific poisons can help detective to understand the situation better.

EXERCISE

1. Multiple Choice Questions (MCQs)

i. In a scenario where a person may have died from a drug overdose at a crime scene, how can mass spectrometry contribute to determining the cause of death?

a) Mass spectrometry can detect the presence of a toxin but not specify which toxin.

b) Carpet fiber analysis can be utilized to match fibers found on the victim.

c) Tissue samples can be examined to identify both the presence and quantity of toxins.

d) Mass spectrometry can ascertain the presence of a toxin but not quantify it.

ii. Detecting amino acids by spraying the plate with ninhydrin solution exemplifies which type of chromatography?

a) Column chromatography
b) Thin layer chromatography
c) Paper chromatography
d) Liquid chromatography

iii. What does the retardation factor represent?

a) The ratio of the distance traveled by the substance from the baseline to the distance traveled by the solvent from the baseline.

b) The ratio of the distance traveled by the solvent from the baseline to the distance traveled by the substance from the baseline.

c) The ratio of the distance traveled by the substance from the top line to the distance traveled by the solvent from the top line.

d) The ratio of the distance traveled by the solvent from the top line to the distance traveled by the substance from the top line.

iv. In chromatography, the stationary phase may be __________ supported on a solid.

a) Solid or liquid
b) Liquid or gas
c) Solid only
d) Liquid only

v. *Which type of* chromatography involves separating substances in a mixture over a 0.2mm thick layer of an adsorbent?

a) Gas liquid
b) Column
c) thin layer
d) Paper

vi. What is the distance that the solute moves while undergoing one partition?

a) Retention distance
b) Distribution constant
c) Plate height
d) Column packing length

vii. In TLC substances are separated because of differences in_______.

a) polarity
b) molecular size
c) concentration
d) electronegativity

viii. Which chromatographic technique is best suited for separating volatile compounds based on their interaction with a stationary phase?

a) Paper chromatography
b) Thin-layer chromatography (TLC)
c) Gas chromatography (GC)
d) High-performance liquid chromatography (HPLC)

ix. Which chromatographic technique is often used for monitoring the progress of reactions and identifying compounds based on their retention factors?

a) Paper chromatography
b) Thin-layer chromatography (TLC)
c) Column chromatography
d) Gas chromatography (GC)

2. Short Answer Questions

i. What causes colors to separate during chromatography?
ii. Is it possible for the R_f value to exceed 1?
iii. Explain the primary use of thin-layer chromatography (TLC)?
iv. Differentiate between thin-layer chromatography (TLC) and paper chromatography?
v. What do "stationary phase" and "mobile phase" refer to in chromatography?
vi. What type of chromatography is used in forensic science?
vii. Describe how you would analyze the chromatograms to determine if two ink samples are from the same source.
viii. Explain the importance of the solvent choice in chromatography and how it affects the separation process.
ix. Assess the limitations of paper chromatography when used to separate complex mixtures?
x. What are the essential characteristics of the substance used as a developer?

3. Long Answer Questions

i. Is mass spectrometry quantitative or qualitative? Give a reason to support your answer.

ii. Evaluate the effectiveness of chromatography as a method for environmental monitoring of pollutants. What are the strengths and weaknesses in this application?

iii. Design an experiment to investigate the effectiveness of different chromatographic techniques in separating and identifying components of a mixture. Evaluate the experimental design, including the choice of chromatographic techniques, sample preparation methods, detection systems, and data analysis strategies. Discuss the potential challenges and limitations of the experiment.

THINK TANK

Group Activity

Teacher will divide the students into groups and Participants will use paper chromatography to solve a crime scene investigation by identifying and comparing ink samples from different suspects. Through this activity, participants will develop a practical understanding of chromatography techniques, enhance their problem-solving skills, and apply their knowledge in a forensic science context.

Outline for activity:

1. Recall basic concepts and principles of chromatography.
2. Explain the scenario where ink samples are found
3. Sample Preparation of chromatography strips
4. Analyze the separation process of ink components.
5. Evaluate and compare chromatograms to identify the matching ink sample.
6. Create a logical conclusion based on chromatographic analysis.
7. Evaluate the entire process.

GROUP PROJECT

Describe a scenario in which the results of a chromatography experiment could be misinterpreted. How would you ensure an accurate interpretation of chromatographic data?