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Class 12
Chemistry

Chapter 19
Chromatography
Handwritten Notes

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CHROMATOGRAPHY

Chromatography is an important laboratory technique used for the separation of mixtures into their individual components. It has wide applications in pharmaceutical, food, chemical, environmental, forensic, and drug-testing industries. It helps scientists identify substances, check the purity of compounds, analyze medicines, test water quality, and study the nutritional content of food. The term chromatography is derived from greek words meaning color and to write, as the technique often separates components in the form of visible colored bands. One major advantage of chromatography is its ability to separate complex mixtures and provide detailed information about the composition and purity of substances.

Main Components of Chromatography

Chromatography is based on the interaction between two phases: a stationary phase and a mobile phase. Separation occurs because different components of a mixture move at different speeds due to their varying affinities for these two phases.

- **Stationary Phase:** The stationary phase is the phase that remains fixed during the chromatographic process. It is usually a solid, such as silica or alumina, or a liquid coated on the surface of a solid. In techniques like column chromatography, the stationary phase is packed inside a column while the mobile phase passes through it. Different components of a mixture interact with the stationary phase to different extents causing separation.
- **Mobile Phase:** The mobile phase is the phase that moves over the stationary phase. It can be a liquid or a gas. In liquid chromatography, the mobile phase is a liquid, while in gas chromatography, it is a gas. As the mobile phase flows through or over the stationary phase, it carries the components of the mixture, which separate based on how strongly they are attracted to the stationary phase.
- **Rf (retardation factor) value:** The Rf value is an important parameter used in chromatography to identify compounds. It is defined as the ratio of the distance traveled by the solute (compound) to the distance traveled by the solvent front from the baseline.
  Rf value = distance traveled by solute / distance traveled by solvent front.
  Each compound has a characteristic Rf value under specific conditions, which helps in identification and purity analysis.
- **Solvent Front:** The solvent front is the furthest point reached by the solvent as it moves through the stationary phase. It indicates how far the mobile phase has traveled during the chromatographic process. Separation occurs due to a dynamic equilibrium between the mobile and stationary phases as components continuously move between them.
- **The baseline:** The baseline is the refrence line drawn near the bottom of the chromatogram where the sample is initially applied. The movement of all components and the solvent front is measured from this line. It serves as the starting point for calculating Rf values.

Thin-Layer Chromatography

Thin-layer chromatography (TLC) is a simple and widely used chromatographic technique for separating components of a mixture based on their different interactions with a stationary surface. In TLC, the stationary phase in a thin layer of adsorbent such as silica gel or alumina coated on a glass plate. The mixture to be analyzed is applied as a small spot about 2cm above the bottom of the plate. When the plate is placed in a closed chamber containing a solvent called the eluent, the solvent rises upward by capillary action and carries the components of the mixture of different heights. Substances that interact strongly with the stationary phase move slowly, while others move faster, producing separate spots. TLC is one of the fastest simplest, and least expensive chromatographic techniques. Pre-made TLC plates are chemically inert, stable, and have a uniformly thin stationary layer. The development chamber provides a controlled environment, prevents solvent evaporation, and keeps the plate free from dust. The mobile phase used in TLC may be a single solvent or a mixture of solvents and should be pure free

clear and well-defined spots.

- **Working of thin layer chromatography:** First, the stationary phase is coated on the TLC plate using silica gel or aluminium oxide anhydride. Small pencil marks are made near the bottom of the plate, and sample solutions are applied on these marks using a capilliary tube. The mobile phase is poured into the TLC chamber, and a moistened filter paper is added to maintain constant humidity. The plate is then placed carefully inside the chamber and covered with a lid. The solvent rises up the plate while keeping the sample spots above the solvent level. After the spots are developed, the plate is removed and dried. The separated spots are usually observed under UV light.
- **Applications of thin layer chromatography:** Thin-layer chromatography is used in testing medicines such as sedatives and local anesthetics. It is useful in biochemical analysis for separating substances obtained from food. TLC helps in identifying natural products like essential oils and alkaloids. It is used for purifying samples and comparing them with standard compounds. In the food industry, TLC is applied for separating and identifying colors, sweeteners, and preservatives. It is also used in the cosmetic industry and in organic synthesis.
- **Limitations of thin layer chromatography:** The separation length in TLC is shorter compared to other chromatographic techniques. Since TLC is an open system, factors such as temperature and humidity can affect the results. This method is mainly suitable for detecting small quantities of substances.
- **Application of thin layer chromatography in forensic chemistry:**

Drug Analysis: Thin-layer chromatography is widely used to separate and identify illegal drugs such as cocaine and heroin. The results are comparad with standard samples to determine the composition and purity of the drugs.
Poison Detection: In suspected poisioning cases, TLC is used to analyze biological samples like blood, urine, and tissues to identify toxic substances.
Trace Evidence Analysis: TLC helps in examining fibers, paints, and dyes found at crime scenes. By comparing these with reference samples, forensics experts can link evidance to a specific source.
Gunshot residue analysis: TLC helps in examining and is used to detect and analyze gunshot residue collected from suspects or crime scenes.
Explosive residue analysis: TLC helps in detecting explosives materials by separating compounds such as nitrates and nitroaromatic substances from forensic samples.
Document examination: TLC is applied in questioned document cases to analyze inks and paper coatings, helping in authentication and detection of forgies.
Preliminary toxicology screening: TLC serves as an initial screening techniques in forensic laboratories to identify drugs in biological samples before detailed analysis using advanced methods like GC or HPLC

Selection of Mobile and Stationary phase:

The selection of suitable stationary and mobile phases is essential for achieving effective separation in chromatography. Proper selection ensures that different components of a mixture move at different rates, resulting in clear and accurate separation.

- **Selection of stationary phase:** In chromatography, the stationary phase generally acts as an adsorbent. It should have a high and selective adsorption capacity so that different components interact with it to different extents. The stationary phase must be finely divided to provide a large surface area for adsorption and should possess high mechanical stability to reduce dust formation. It should be chemically inert toward the sample and the eluting solvent to avoid unwanted reactions. High purity of the stationary phase is necessary for accurate results, and it should be easily available.

The stationary phase is selected in such a way that the components of the mixture show different solubilities or affinities toward it. As a result, the components move at different speeds and become separated. For separating closely related compounds with high resolution, a stationary phase with small particle size is preferred, as it increases interaction with the components and improves separation efficiency.

- Selection of mobile phase in chromatography: The selection of the mobile phase plays a key role in successful chromatographic separation. The solvent used should be of high purity, preferably HPLC- or MS-grade, to prevent impurities from interfering with the analysis. Solvents should be obtained from reliable manufacturers with good quality control.
Polarity of the mobile phase should match the nature of the analyte for effective interaction and movement. The viscosity of the solvent should be appropriate to maintain a proper flow rate and good peak shape. The mobile phase must be chemically stable and non-reactive toward both the analyte and the stationary phase. If UV detection is used, the solvent should be transparent at the detection wavelength. In cases involving charged species, control of pH is very important to ensure proper separation.

SHORT QUESTIONS

What causes colors to separate during chromatography?
Colors separate during chromatography because different components of a mixture have different affinities for the stationary and mobile phases. Some components are more strongly adsorbed on the stationary phase and move slowly, while others move faster with the mobile phase. This difference in movement causes separation.
Is it possible for the Rf value to exceed 1?
No, the Rf value cannot exceed 1. This is because the distance traveled by the solute can never be greater than the distance traveled by the solvent front. Therefore, the maximum possible value of Rf is 1.
Explain the primary use of thin-layer chromatography.
The primary use of thin-layer chromatography is to separate and identify components of a mixture. It is widely used to check purity, identify compounds, and compare samples. TLC is also used in pharmaceutical, food, and forensic analysis.
What do stationary phase and mobile phase refer to in chromatography.
The stationary phase is the fixed phase that does not move and is usually a solid adsorbent like salica gel or alumina. The mobile phase is the liquid or gas that moves over the stationary phase and carries the components of the mixture. Separation occurs due to different interactions of components with these two phases.
What type of chromatography is used in forensic science?
Thin-layer chromatography is commonly used in forensic science. It helps in analyzing drugs, poisons, inks, explosive residues, and trace evidance. TLC provides quick and reliable preliminary analysis of forensic samples.
Describe how you would analyze the chromtograms to determine if two ink samples are from the same source.
The ink samples are applied on the same TLC plate and developed under identical conditions. If both samples produce the same number of spots with identical Rf values and positions, they are likely from the same source. Differences in spots indicate different ink compositions.
Explain the importance of the solvent choise in chromatography and how it affects the separation process.
The choice of solvent is important because it controls the movement of components on the stationary phase. A solvent with suitable polarity ensures proper interaction and effective separation. Solvent purity, viscosity, chemical stability, and pH also affect the quality of separation.