**Introduction, methodology of paper chromatography**

Introduction:

Paper chromatography is a widely used technique in analytical chemistry for separating and identifying components of a mixture based on their differential migration through a stationary phase (paper) by capillary action. It is a simple, inexpensive, and effective method that requires minimal equipment, making it suitable for qualitative and quantitative analysis of various substances. Paper chromatography finds applications in fields such as pharmaceuticals, forensics, environmental monitoring, and food analysis.

Methodology of Paper Chromatography:

1. Selection of Paper and Stationary Phase:
   * Choose a suitable chromatography paper with good capillary action and uniform composition, such as filter paper or Whatman paper.
   * Ensure that the paper is free from contaminants or impurities that may interfere with the separation process.
   * Prepare the stationary phase by applying a thin layer of suitable adsorbent or chromatography solvent (e.g., silica gel, cellulose) onto the paper's surface. This creates the stationary phase where separation occurs.
2. Preparation of Sample and Mobile Phase:
   * Prepare the sample to be analyzed by dissolving or suspending it in a suitable solvent. Ensure the sample is free from impurities or particulate matter that may affect the separation.
   * Prepare the mobile phase or eluent, which is the solvent that moves through the paper and carries the sample components. The choice of mobile phase depends on the sample characteristics and desired separation. Common mobile phases include water, ethanol, or mixtures of organic solvents.
3. Spotting the Sample:
   * Apply the prepared sample onto the chromatography paper as a small spot or line near one end, using a capillary tube or micropipette.
   * Allow the sample spot to dry completely to prevent smearing during the subsequent steps.
4. Developing the Chromatogram:
   * Set up a chromatography chamber by placing the paper vertically in a solvent-resistant container.
   * Pour the mobile phase into the chamber, ensuring that the solvent level is below the sample spot to avoid dissolving the sample directly.
   * Cover the chamber to minimize solvent evaporation and allow the solvent vapor to saturate the atmosphere inside the chamber.
   * Let the chromatogram develop by capillary action as the solvent moves up the paper. The different components of the sample will separate into distinct bands based on their solubility and affinity for the stationary phase.
5. Visualization and Analysis:
   * After the desired solvent front has reached the top of the paper (usually around two-thirds of the paper height), remove the paper from the chamber.
   * Mark the solvent front with a pencil and allow the paper to dry completely.
   * Visualize the separated components by treating the paper with suitable detection techniques, such as UV light, iodine vapor, or chemical reagents specific to the components of interest.
   * Measure the distance traveled by each component (Rf value), which is the ratio of the distance traveled by the component to the distance traveled by the solvent front.
   * Compare the obtained Rf values with known standards or literature values to identify the separated components.
6. Documentation and Interpretation:
   * Record the experimental details, including the sample, mobile phase, paper used, and any observations or unexpected results.
   * Analyze the chromatogram to interpret the separation patterns, identify the components, and quantify their relative amounts if applicable.
   * Draw conclusions based on the separation efficiency, resolution, and the relationships between the identified components.

It is important to note that the methodology of paper chromatography can be adjusted based on the specific requirements, sample characteristics, and desired separation goals. Optimization of the mobile phase composition, paper selection, and spotting techniques may be necessary to achieve the desired separation and resolution