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# A BRIEF REVIEW ON DIFFERENT CHROMATOGRAPHY TECHNIQUES

**Harshad Harishchandra Ubale*, Bhalekar Onkar Dashrath, Chaudhari Swapnil Raghunath, Pingle Tejas Mahadev, Arman Altaf Atar**

Student Fabtech College of Engineering Sangola Solapur Maharashtra India.

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***Corresponding Author**
**Harshad Harishchandra Ubale**
Student Fabtech College of Engineering Sangola
Solapur Maharashtra India.

## ABSTRACT

A common approach of distinguishing components in a mixture is chromatography. Differential partitioning between the mobile and stationary phases has for component separation. This review offers a succinct synopsis of the many chromatography methods, emphasizing their developments, applications, and guiding principles. There is discussion of the three main GC is a very useful technique for separating volatile compounds because it takes advantage of variations in the volatility of the compounds and their interactions with the stationary phase. Because it offers high resolution and sensitivity, liquid chromatography, in particular HPLC is widely used for non- volatile & thermally unstable compounds. TLC is an easier and less expensive method that is used as a first step in the identification and purity assessment of compounds. The review also discusses cutting-edge methods such as Affinity Chromatography and Supercritical Fluid Chromatography (SFC), which have specific uses in fields like biomolecule purification and chiral separations, respectively.

**KEYWORDS:** Chromatography, Chromatography Techniques, TLC.

## INTRODUCTION

In order to separate, identify, and quantify the constituents of complex mixtures, chromatography is a fundamental analytical technique that is widely used in the fields of chemistry, biology, and environmental science. Chromatography was first used in the early 1900s and has since expanded into a wide range of techniques,

each suited to a particular class of analytes and use. Its accuracy and adaptability have made it a mainstay in a variety of industries, including biochemical research, environmental monitoring, and pharmaceutical development. The various chromatography methods are examined in this review, each with specific principles and applications.[1] While liquid chromatography is crucial for the analysis of non-volatile and thermally labile substances, GC is especially useful for the analysis of compounds. Rapid qualitative analysis can be accomplished more quickly and effectively with thin layer chromatography.[2]

**Definition:** A laboratory technique that separates components in a mixture by determining how distinctively each component interacts with a mobile phase & a stationary phase. By taking advantage over variations in the components' solubility, volatility, or affinity for the stationary phase, the method differentiates the constituents as they move through the system at multiple speeds.[3]

**Principle:** Differential partitioning, which divides a mixture's components differently they interact with two different phases— stationary phase & mobile phase—is the foundation of chromatography.

1. **Stationary phase:** Viscous liquid that is immobilized inside the chromatography system is called stationary phase. It can be affixed to the inside walls of a tube, packed into a column. The ability of the stationary phase to interact with the mixture's constituents by a variety of mechanisms, including adsorption, partitioning, and ion exchange, is what defines it.[4]

2. **Mobile phase:** Mixture of components are carried out by mobile phase, which is a fluid that passes through a stationary phase. The components flow through the chromatographic system more easily thanks to the mobile phase. Composition & characteristics such as polarity, ph and ionic strength, can change depending on the method used.

3. **Differential interaction:** Each component interacts with mobile & stationary phases of system in a different way as the mixture is added. charge, volatility, and molecular size, polarity among other physical and chemical characteristics, control these interactions.[4] Stronger affinities for the stationary phase will cause

constituents to stick to it more and flow through the system more slowly. Components that are more suited to the mobile phase, on the other hand, will move faster.

4. **Partition coefficient:** Partition coefficient, or the ratio of a component's conc of stationary phase to its concentration of mobile phase, determines how quickly a component moves through system.[5] A lower partition coefficient denotes faster movement through the system, whereas a higher partition coefficient indicates the component is more retained by the stationary phase and moves more slowly.

5. **Separation process:** These differential movements cause the components to separate over time or space. A sequence of divided zones or peaks is produced as each component leaves the system at a different time (for gas or liquid chromatography, at a different place for techniques like TLC). The choice mobile & stationary phases, temperature, flow velocity, and physical properties of the components being separated are some of the variables that affect the degree of separation.

6. **Detection and Analysis:** Following their separation, the components are usually found using a variety of detectors (such as mass spectrometry and UV-Vis spectroscopy) that gauge each component's concentration as it leaves the system. This makes it possible to determine and measure each of the mixture's constituent parts.[6]

**Chromatography techniques**

1. **Gas chromatography**

**Principle:** Based on how volatile substances are distributed betn mobile & stationary phase—an inert gas—it separates them (typically helium or nitrogen).

**Components:** Stationary Phase - usually a solid particulate pack or polymer coated inside long, narrow column (capillary column).

**Mobile phase:** An inert gas that transports the vaporised analyte through column.[7]

**Process:** In an injection port sample is vaporised, & mobile phase then transfee it through stationary phase. Separation results from components interacting with the

stationary phase according to their polarity and volatility. Lower boiling point components or those that interact with the stationary phase less strongly move through the elute process more quickly.

**Applications:** Volatile organic substances, environmental contaminants, essential oils, and tastes are all frequently analyzed using gas chromatography (GC). Additionally, the petrochemical industry and forensic science depend on it.[8]

**2. Liquid chromatography**

**Principle:** With liquid chromatography, components are separated and well they bind to the stationary phase as opposed to mobile phase of liquid

**Types**

**HPLC:** Widely developed type LC that accelerates analysis and improves separation by forcing mobile phase (MP) through a tightly packed column under high pressure.[9]

**Liquid Chromatography at Low Pressure (LPLC):** A more straightforward type of LC that works at lower pressures and is usually employed in preparation.

**Components**

**Stationary phase:** Typically a densely packed column of particles covered in a particular substance (Silica gel, for example) that interacts with the constituents of the sample.

**Mobile phase:** liquid solvent is chosen based on the sample's properties and the desired separation.[10]

**Process:** liquid analyte is esta into the column & carried out through column of the mobile phase. Based on their interactions (e.g., hydrophobicity, polarity) with the stationary phase, components separate. Components that are more firmly maintained elute later.

**Applications:** Non-volatile or thermally unstable substances, such as medications, biomolecules (proteins, peptides), and environmental samples, can be analyzed and purified using LC. HPLC is especially crucial for quality assurance and medication development.[11]

### 3. Thin layer chromatography

**Principle:** Using capillary action to move stationary & mobile phase coated on flat surface, TLC separates components according to their affinity.

**Components**

**Stationary phase:** A thin layer of adsorbent material coated on a glass, plastic, or aluminum plate.

**Mobile phase:** Analayte that moves up the plate by capillary action.

**Process:** Near the TLC plate's bottom, a tiny area of the sample is applied. the plate is put in a developing chamber containing the mobile phase. The sample's constituent parts are carried on plate by a mobile phase. Different migration distances are produced by components separating according to their varying affinities for the stationary phase.[12]

**Applications:** TLC is used for rapid qualitative analysis, including chemical identification, drug purity checks, and reaction progress monitoring. It is frequently used in forensic research, medicines, and chemical synthesis.

### 4. Ion exchange chromatography

**Principle:** Based on their affinity for an ion exchanger, which is the stationary phase, IEC separates ions and polar molecules.

**Components**

**Stationary phase:** A resin or polymer with charged functional groups that interact with opposing charged ion in analyte.

**Mobile phase:** Aq. buffer that can vary in pH, ionic strength, or composition to facilitate separation.[13]

**Process:** Ions in the sample interact with the oppositely charged groups on the stationary phase when it is inserted into the column. According to their charge and degree of interaction with the ion exchanger, components are divided into several groups. The mobile phase's pH or ionic strength can be adjusted to produce elution.

**Applications:** IEC is a commonly employed technique in purification of charged

proteins and nucleic acids (biomolecules). It is also used in the inorganic ion separation process and water purification.[14]

### 5. Size exclusion chromatography

**Principle:** It separates molecules on the basis of size by passing them through a porous stationary phase.

**Components**

Stationary phase: A column packed with porous beads that create a size- dependent path for molecules.[15]

**Mobile phase:** An inert solvent that transports the sample through the column.

**Process:** Smaller molecules access the beads' pores and take longer to elute than larger ones, which are prevented from doing so as the material passes through the column.

**Applications:** Proteins, polymers, and other macromolecules are analysed and purified using SEC. For figuring out molecular weight distributions, it is quite helpful.[16]

### 6. Affinity chromatography

**Principle:** Biomolecules are separated using affinity chromatography according to certain interactions between the target molecule and a ligand bound to the stationary phase.

**Components**

**Stationary phase:** A resin or matrix with a specific ligand (e.g., antibody, enzyme, or substrate) that selectively binds to the target molecule.

**Mobile phase:** A buffer solution that facilitates the binding and elution of the target molecule.[17]

**Process:** The ligand-containing column is passed through with the sample. Other components are removed while the target molecule attaches to the ligand. After the binding is broken, the target molecule is eluted by adjusting the parameters (pH, ionic strength, etc.).

**Applications:** Continuity Purifying proteins, antibodies, enzymes, and other biomolecules is a common application of chromatography. It is also used in the isolation of particular cell types and the research of molecular interactions.[18]

**7. Supercritical Fluid Chromatography (SFC)**

**Principle:** SFC separates components according to their solubility and interactions with the stationary phase by using a supercritical fluid (often CO2) as the mobile phase.

**Components**

**Stationary phase:** Similar to HPLC, often packed with silica or other materials compatible with supercritical fluids.

**Mobile phase:** A component at a temp & pressure above to critical point called supercritical fluid, can display characteristics of both a liquid and a gas.[19]

**Process:** After the analyte is inserted to column, it is transfer through stationary phase by the supercritical fluid. Components with different polarity and molecular weights can be effectively separated thanks to the special qualities of the supercritical fluid.

**Applications:** SFC is utilised in the separation of complicated natural goods, medicines, and chiral chemicals. Due to its effectiveness and low environmental impact, it is also used in environmental analysis and food safety.[20]

**8. Paper chromatography**

**Principle:** Components are separated via paper chromatography according to how well they dissolve mobile phase and cling to cellulose stationary phase.

**Components**

**Stationary phase:** Sheet of the cellulose-based paper that acts as the adsorbent.[21]

**Mobile phase:** A analyte that moves through the paper by capillary action.

**Process:** Edge of paper is positioned in mobile phase after a tiny spot of the sample is put to it. The components of the sample are carried out solvent as it passes through the paper. The way that different components interact with the cellulose

fibres and how soluble they are in the solvent cause them to separate.[22]

**Applications:** Paper chromatography is an easy-to-use and reasonably priced technology that is frequently employed in educational settings for pigment separation as well as in some biological applications, like nucleotide and amino acid separation.

These techniques continue to be at the forefront of analytical science thanks to the constant improvements in chromatographic technologies.[23]

## CONCLUSION

Chromatography is an analytical technique that is essential and has many applications. Over time, it has developed into a set of techniques with specialised applications for separating, identifying, and quantifying complex mixtures. Affinity Chromatography stands out for its specificity in isolating target molecules based on biological interactions, whilst IEC & SEC address specialised needs of biomolecule purification and polymer analysis, respectively.

The wide range of chromatography techniques guarantees their applicability in numerous scientific and industrial domains, such as food safety, biotechnology, environmental research, and pharmaceuticals. Chromatography is still a vital instrument in the modern analytical laboratory, offering crucial insights into the behaviour and composition of complex substances, even as technological developments improve its sustainability, efficiency, and resolution.

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