# CHROMATOGRAPHY

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## I. INTRODUCTION TO CHROMATOGRAPHY

Chromatography is a powerful analytical technique employed to separate and purify the constituents of a mixture. The term "chromatography" is derived from the Greek words "chroma" (color) and "graphein" (to write), reflecting its historical application in separating colored pigments. This method involves a stationary phase (solid or liquid) and a mobile phase (liquid or gas). The mixture is introduced into the stationary phase, and the mobile phase carries the components at different rates based on their interactions with both phases.

## II. HISTORICAL PERSPECTIVE

Mikhail Tswett, a Russian botanist, coined the term "chromatography" in 1906. While its initial applications focused on plant pigments, the technique's analytical potential was recognized by Archer John Porter Martin and Richard Laurence Millington Synge, who introduced gas chromatography in 1952 for analysing fatty acid mixtures.

## III. PRINCIPLE

Chromatography exploits the differential partitioning of components between the stationary and mobile phases. Differences in properties such as size, polarity, charge, and affinity influence the interaction of components with the stationary phase, leading to varying retention times. As the mobile phase carries

the components through the system, they are detected, and the resulting signal is recorded as a chromatogram.

## IV. KEY COMPONENTS

Chromatography relies on three essential components:

1. **Stationary Phase:** A solid or liquid phase that remains fixed within the chromatographic system.

2. **Mobile Phase:** A liquid or gas that moves through the stationary phase, carrying the components of the mixture.

3. **Separated Molecules:** The components of the mixture that are separated based on their interactions with the stationary and mobile phases.

### Rf VALUE

The Rf value, or retention factor, is a characteristic parameter used to identify compounds in chromatography. It represents the ratio of the distance traveled by an analyte to the distance traveled by the solvent front.

## V. TYPES OF CHROMATOGRAPHY

Several chromatographic techniques exist, each exploiting different separation principles:

1. **Paper Chromatography:** Utilizes paper as the stationary phase for separating dissolved compounds based on differential migration rates.

2. **Thin-Layer Chromatography (TLC):** Similar to paper chromatography but employs a thin layer of adsorbent on a solid support.

3. **Column Chromatography:** Employs a packed column as the stationary phase for separating components based on adsorption, partition, or size exclusion principles.

    - **Ion Exchange Chromatography:** Separates ions based on their charge interactions with the stationary phase.

- **Gel Filtration Chromatography:** Separates molecules based on size differences.

4. **Affinity Chromatography:** Separates molecules based on specific binding interactions with a ligand immobilized on the stationary phase.

5. **Gas-Liquid Chromatography (GLC):** Separates volatile compounds based on their partitioning between a gaseous mobile phase and a liquid stationary phase.

6. **High-Performance Liquid Chromatography (HPLC):** A versatile technique using high pressure to force a liquid mobile phase through a packed column, enabling rapid and efficient separations.

## VI. PAPER CHROMATOGRAPHY

### Principle

Paper chromatography operates on either partition or adsorption principles. In partition chromatography, components distribute between the water retained in the paper and the mobile phase. In adsorption chromatography, the paper's solid surface acts as the stationary phase. The differential affinity of components for the two phases, combined with capillary action, leads to separation.

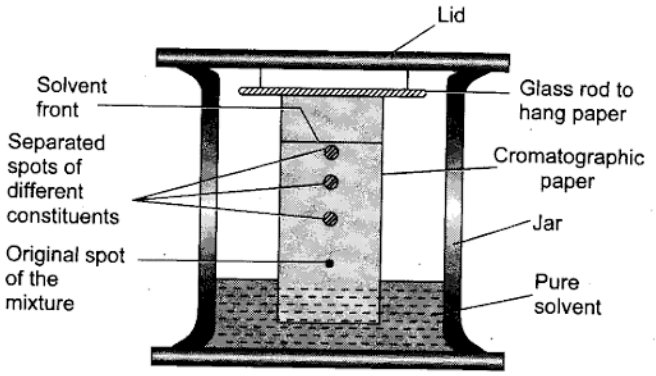


**Figure 1:** Paper Chromatography

### Procedure

Paper chromatography is a technique used to separate components of a mixture based on their differential adsorption on a stationary phase (paper) and their varying solubility in a mobile phase (solvent).

## VII. STEPS INVOLVED

1. **Choice of Development Method:** The selection of a development method depends on factors such as the solvent, paper type, and desired outcome. Ascending or radial chromatography is often preferred due to its simplicity and rapid results.

2. **Filter Paper Selection:** The appropriate filter paper is chosen based on pore size and sample characteristics.

3. **Sample Preparation:** The sample is dissolved in a suitable solvent that is inert to the components being analyzed. This solution becomes the mobile phase.

4. **Sample Application:** The prepared sample is carefully spotted onto a specific location on the filter paper using a capillary tube.

5. **Chromatogram Development:** The spotted paper is immersed in the mobile phase. Capillary action draws the solvent up the paper, carrying the sample components with it.

6. **Drying and Visualization:** Once the solvent front reaches a desired height, the paper is removed and dried. Visualization techniques, such as spraying with a detecting reagent, are employed to locate the separated components.

### Applications

Paper chromatography has diverse applications:

- Analysis of fermentation and ripening processes
- Assessment of pharmaceutical purity
- Examination of cosmetic formulations
- Detection of adulterants in various substances
- Identification of contaminants in food and beverages
- Analysis of reaction mixtures in biochemical studies
- Detection of drugs and doping agents in humans and animals

## VIII. THIN-LAYER CHROMATOGRAPHY (TLC)

TLC is another chromatographic method used to separate non-volatile mixtures. It involves a thin layer of adsorbent material (e.g., silica gel, alumina) coated on a support (e.g., glass, plastic, aluminum foil).

### Principle

TLC operates on the same principle as paper chromatography: differential adsorption of components onto the stationary phase and their varying solubility in the mobile phase. Components with a higher affinity for the stationary phase move slower, resulting in separation.

### Procedure

1. **Sample Preparation:** The sample is prepared as a solution in a suitable solvent.

2. **Spotting:** The sample solution is applied as a small spot on the TLC plate.

3. **Development:** The TLC plate is placed in a developing chamber containing the mobile phase. Capillary action draws the solvent up the plate.

4. **Visualization:** After the solvent front reaches a desired height, the plate is removed, dried, and developed using appropriate visualization techniques.

## IX. RETENTION FACTOR (Rf)

The Rf value is a characteristic property of a compound in a specific TLC system. It is calculated as:

Rf = Distance traveled by the sample / Distance traveled by the solvent

The Rf value is influenced by factors such as the solvent system, adsorbent, temperature, and amount of sample applied.

TLC is a rapid, inexpensive, and simple technique widely used in various fields for qualitative and quantitative analysis.

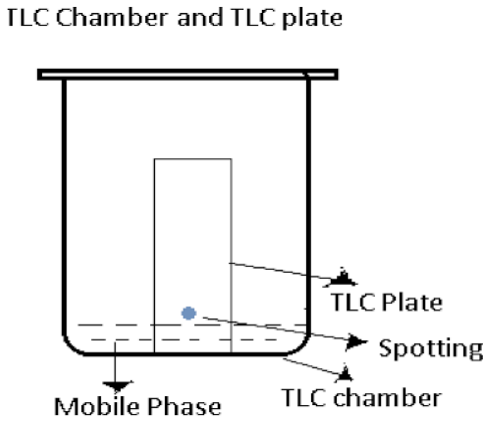


**Figure 2:** Diagram of Thin Layer Chromatography

## X. COMPONENTS AND PROCEDURE

1. **Stationary Phase:** A TLC plate consists of a chemically inert and stable support coated with a thin, uniform layer of adsorbent particles. This adsorbent, often silica gel or alumina, interacts with the components of the mixture to be separated.

2. **Mobile Phase:** A suitable solvent or solvent mixture is used as the mobile phase. The choice of solvent is crucial as it affects the separation of components based on their solubility and affinity for the stationary phase.

3. **TLC Chamber:** A closed container that maintains a consistent environment for the development of chromatograms. It prevents solvent evaporation and minimizes external disturbances.

4. **Filter Paper:** Placed in the TLC chamber to saturate the atmosphere with solvent vapors, ensuring even development of the chromatogram.

### Procedure

1. **Sample Application:** A small amount of the sample mixture is applied as a spot near the bottom of the TLC plate using a capillary tube.

2. **Development:** The TLC plate is placed in a TLC chamber containing the mobile phase. The solvent ascends the plate by capillary action, carrying the components of the mixture with it.

3. **Visualization:** After the solvent front reaches a desired height, the plate is removed, dried, and visualized. Components are identified based on their retention factors (Rf values) and comparison with known standards.

### Applications

TLC has diverse applications in various fields:

1. **Pharmaceutical Analysis:** Identification and quantification of drugs, impurities, and degradation products.

2. **Biochemical Analysis:** Separation and analysis of biomolecules such as amino acids, lipids, and carbohydrates.

3. **Natural Product Analysis:** Isolation and characterization of compounds from plants and other natural sources.

4. **Food Analysis:** Detection of additives, contaminants, and quality control.

5. **Forensic Science:** Analysis of drugs, explosives, and other forensic samples.

### Limitations of TLC

1. **Qualitative Analysis:** Primarily used for qualitative analysis, providing limited quantitative information.

2. **Sensitivity:** Less sensitive compared to other chromatographic techniques.

3. **Reproducibility:** Can be challenging to achieve consistent results due to factors like temperature, humidity, and plate quality.

## XI. COLUMN CHROMATOGRAPHY

Unlike TLC, column chromatography involves a vertical glass column packed with an adsorbent material. The sample mixture is applied to the top of the column, and a solvent is gradually passed through. Components are separated based on their affinity for the stationary phase and are collected as separate fractions.

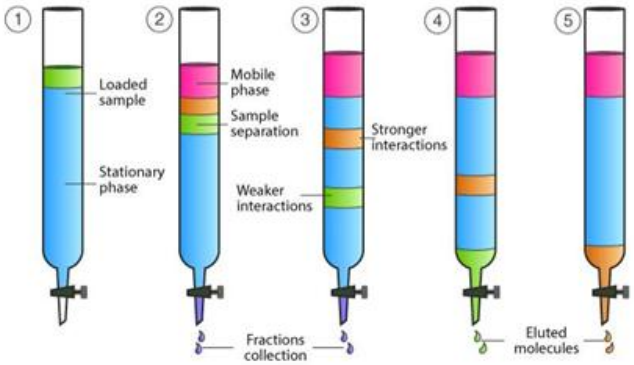


**Figure 3:** Column Chromatography

## Principle

Column chromatography uses a stationary solid phase to adsorb and separate substances that pass through it, which is assisted by a liquid mobile phase. Compounds are adsorbed depending on their chemical composition, and elution is determined by the adsorbent's differential adsorption of a material.

## XII. ION EXCHANGE CHROMATOGRAPHY

Ion Exchange Chromatography (or ion chromatography) is a method that separates ions and polar compounds based on their affinity for ion exchangers. The concept of separation is thus based on the reversible exchange of ions between the target ions in the sample solution and the ions on the ion exchanger.

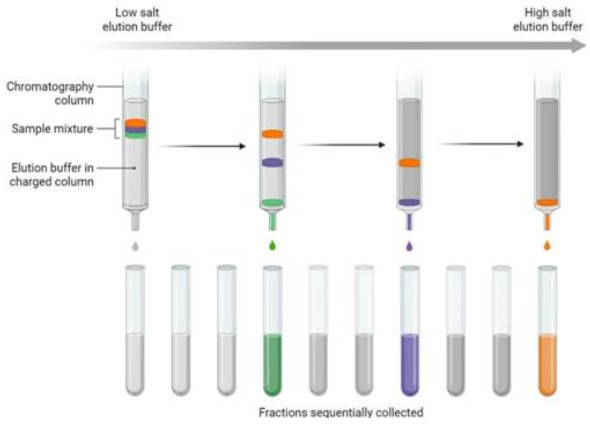


**Figure 4:** Ion Exchange Chromatography

## XIII. ION EXCHANGERS

There are two primary types of ion exchangers:

1. **Cation Exchangers:** These possess negatively charged functional groups, which attract positively charged ions (cations). Due to the acidic nature of these functional groups, they are often referred to as acidic ion exchangers.

2. **Anion Exchangers:** These contain positively charged functional groups, which attract negatively charged ions (anions). Consequently, they are termed basic ion exchangers.

### Principle

IEC operates on the principle of electrostatic attraction between the oppositely charged ions in the sample and the ion exchanger. The strength of this interaction depends on factors such as the charge of the ions, the ionic radius, and the pH of the mobile phase. The ions in the sample compete with ions in the mobile phase for binding sites on the ion exchanger. This competition results in differential retention of ions, allowing for their separation.

### Instrumentation

A typical IEC system comprises the following components:

1. **Pump:** Delivers a constant flow of mobile phase through the system.

2. **Injector:** Introduces the sample into the system.

3. **Column:** Contains the ion exchange stationary phase.

4. **Suppressor:** Improves detector sensitivity by reducing the background conductivity of the eluent.

5. **Detector:** Measures the concentration of ions in the eluent. Common detectors include conductivity detectors.

6. **Data system:** Collects and processes the detector signal.

## Procedure

1. **Column Preparation**: The column is packed with the appropriate ion exchange resin based on the charge of the target ions.

2. **Sample Loading**: The sample is introduced into the column, where ions interact with the stationary phase.

3. **Elution**: A suitable eluent is passed through the column to displace the bound ions. The elution conditions, such as pH, ionic strength, and type of eluent, are optimized for effective separation.

4. **Detection**: The eluted ions are detected, and the resulting signal is recorded.

## Applications

IEC finds widespread applications in various fields, including:

- Water purification
- Food and beverage industry
- Pharmaceutical industry
- Environmental analysis
- Biotechnology

By understanding the principles and instrumentation of IEC, researchers and analysts can effectively utilize this technique for the separation and analysis of ionic compounds.

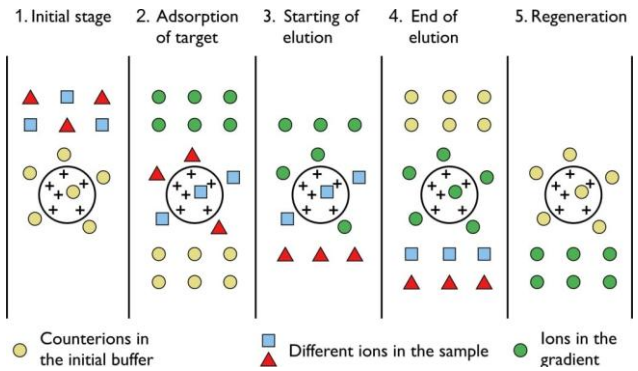


**Figure 5:** Ion Exchange Chromatography

## Applications

- Ion-exchange chromatography is commonly used to analyze amino acid combinations.
- The 20 primary amino acids from blood serum or protein hydrolysis are isolated and utilized in clinical diagnosis.
- This is the most efficient way of water filtration. To completely deionize water (or a non-electrolyte solution), solute cations are exchanged for hydrogen ions and solute anions for hydroxyl ions. This is generally accomplished by a process used for softening drinking water. In the analysis of products of hydrolysis of nucleic acids. In this way, information is gained about the structure of these molecules and how it relates to their biological function as carriers of hereditary information.
- Chelating resins are used to collect trace metals from seawater. To analyze lunar rocks and rare trace elements on Earth.

## Advantage

- It is one of the most efficient methods for the separation of charged particles.
- It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids.
- Ion exchange is used for both analytical and preparative purposes in the laboratory, the analytical uses being the more common.
- Inorganic ions also can be separated by ion-exchange chromatography

# XIV. GEL FILTRATION CHROMATOGRAPHY

Gel Filtration Chromatography separates the samples based on the molecular size and shape of species present in them. It is also called gel permeation chromatography, molecular sieve chromatography, or elution chromatography.

## Principle

Gel filtration chromatography is a method for separating solutes by including and excluding them from a stationary phase. The stationary phase is made out of heteroporous, cross-linked polymeric gel. Separation takes place between the liquid phase within the gel particle and the liquid surrounding it. Molecular diffusion occurs throughout a gel as a result of liquid flow. The bigger molecule appears first, followed by the smaller molecule. The absence of the gel particle creates a size barrier that allows for this separation. Gel chromatography is

carried out in a column using elution. Small molecules diffuse into the gel and travel a longer distance than bigger molecules, which are released from the gel particles. Then compound separation occurs with the large molecules leaving the column first and the small ones last.

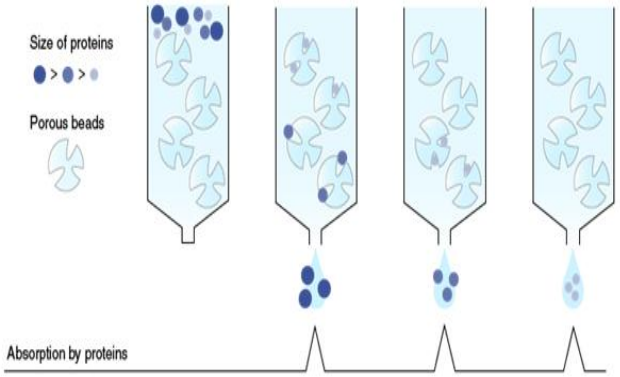


**Figure 6:** Gel Filtration Chromatography

Gel filtration chromatography, also known as size exclusion chromatography, is a technique used to separate molecules based on their size. The process involves a column packed with a gel matrix containing pores of specific sizes. When a mixture of molecules is applied to the column, larger molecules are excluded from the pores and elute first, while smaller molecules enter the pores, travel a longer path, and elute later.

## Gel Matrix

- Composed of polymeric organic molecules with a three-dimensional network of pores.
- Swells in a suitable solvent, creating spaces between the polymer chains.
- Only molecules smaller than the pore size can enter the gel matrix.
- Commonly used gels include dextran, agarose, and polyacrylamide.

## Steps Involved

1. **Column Selection:** Choose a glass column with a fixed cross-sectional area, equipped with a mobile phase reservoir and a bead support at the bottom.

2. **Gel Preparation:** Prepare the gel by swelling dry powder in excess solvent or by heating a slurry of the gel in a water bath.

3. **Column Packing:** Carefully pack the swollen gel into the column, avoiding air bubbles.

4. **Sample Application:** Apply the sample mixture to the top of the gel bed, ensuring it sinks into the gel.

5. **Elution:** Continuously pass solvent through the column to separate the components.

6. **Collection and Analysis:** Collect the eluent in fractions and analyze the components using appropriate methods (e.g., spectrophotometry, colorimetry, chemical tests).

### Applications

- Separation of molecules based on size (e.g., sugars, proteins, nucleic acids).
- Purification of biological macromolecules.
- Concentration of solutions.

### Advantages

- Short analysis time
- Well-defined separation
- High sensitivity
- Low mobile phase volume
- Consistent flow rate

## XV. AFFINITY CHROMATOGRAPHY

Affinity chromatography is a technique that exploits the specific interaction between molecules (e.g., enzyme-substrate, antigen-antibody) to purify and isolate a particular component from a mixture. A ligand, which is a molecule that specifically binds to the target molecule, is immobilized on a solid support. When the sample is passed through the column, the target molecule binds to the ligand, while other components pass through unretarded. After washing away unbound substances, the target molecule is eluted by changing the conditions to disrupt the ligand-target interaction.

### Key Points

- Based on specific biological interactions (affinity).
- Ligand is immobilized on a solid support.
- Target molecule binds to the ligand, while impurities pass through.
- Elution achieved by changing conditions to disrupt the interaction.

### Example

- Purification of an enzyme using a specific inhibitor as the ligand.

### Discovery

- Pedro Cuatrecasas and Meir Wilcheck are credited with the development of affinity chromatography.

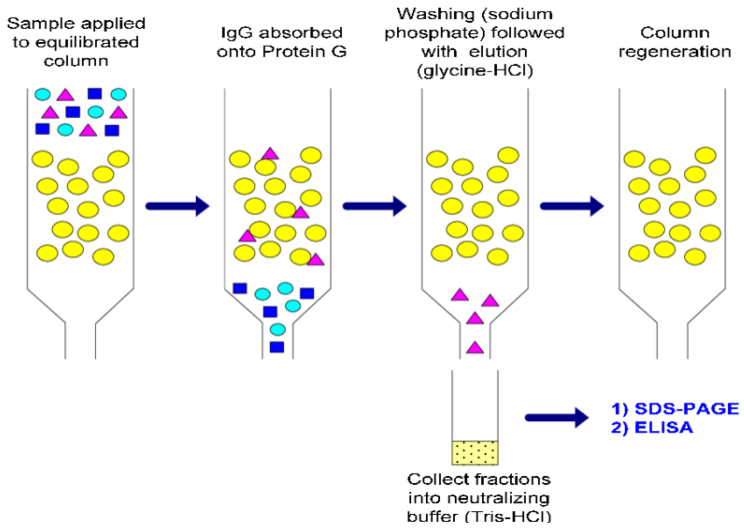


**Figure 7:** Affinity Chromatography

## XVI. AFFINITY CHROMATOGRAPHY: A POWERFUL TOOL FOR PURIFICATION

Affinity chromatography is a versatile technique for separating and purifying specific molecules from a mixture. It leverages highly specific interactions between a target molecule and a complementary molecule, known as a ligand. This targeted approach offers significant advantages over other separation methods.

## The Principle

The core principle of affinity chromatography relies on two key components:

1. **Stationary Phase:** This acts as a solid support, typically made from inert materials like agarose or polyacrylamide. A crucial step involves attaching a ligand to this matrix. The ligand is carefully chosen based on its ability to bind specifically to the target molecule of interest. This attachment can be direct or achieved through a spacer arm that enhances binding efficiency.

2. **Mobile Phase:** This is a liquid containing the sample mixture. When passed through the column packed with the ligand-immobilized stationary phase, only the target molecules will bind to the ligand due to their specific interaction. Unbound components will simply flow through the column and be collected as waste.

## Components

1. **Matrix:** This inert support provides a platform for attaching the ligand.
   - **Key Characteristics Include:** Chemical and physical inertness, insolubility in the employed buffers and solvents, chemical and mechanical stability, ease of coupling to a ligand or spacer, and good flow properties with a large surface area for attachment.

2. **Spacer Arm (Optional):** This flexible molecule bridges the gap between the ligand and the matrix, optimizing binding between the target molecule and the ligand by overcoming steric hindrance effects.

3. **Ligand:** This is the key player in affinity chromatography. It's a molecule that specifically and reversibly binds to the target molecule. The choice of ligand depends on the nature of the target molecule being isolated. Examples include:

   - Hormones for isolating hormone receptor proteins
   - Antigens or haptens for antibody isolation
   - Substrate analogs, inhibitors, cofactors, or effectors for enzyme purification

## Procedure

1. **Column Equilibration:** The column packed with the ligand-immobilized matrix is first equilibrated with a suitable buffer to prepare it for sample loading.

2. **Sample Application:** The sample mixture containing the target molecule is loaded onto the column under conditions that promote selective binding of the target to the ligand.

3. **Washing:** Unbound components are washed away with a buffer, leaving the target molecule bound to the ligand.

4. **Elution:** The specifically bound target molecule is then eluted from the column. This can be achieved by introducing a competitive ligand that displaces the target from the binding site, or by altering the buffer conditions (pH, ionic strength, or polarity) to weaken the target-ligand interaction.

5. **Purification and Concentration:** The eluted target molecule is collected and concentrated as needed. The column can then be re-equilibrated for further use.

## Applications

Affinity chromatography is a powerful tool for various applications, particularly in the field of biochemistry:

1. **Separation and Purification:** This is its primary application, allowing for the isolation of highly specific molecules from complex mixtures.

2. **Enzyme Assays:** Affinity chromatography can be used to isolate and purify enzymes for activity studies.

3. **Substrate Detection:** Specific enzymes can be immobilized on a column to identify their corresponding substrates.

4. **Binding Site Investigation:** Affinity chromatography aids in studying the binding sites of enzymes and other biomolecules.

5. **Antigen-Antibody Interactions:** The technique can be employed to study interactions between antigens and antibodies in vitro.

6. **Nucleic Acid Analysis:** Affinity chromatography finds application in detecting single nucleotide polymorphisms (SNPs) and mutations in nucleic acids.

**Advantages**

1. **High Specificity:** Affinity chromatography offers unmatched specificity, allowing for the isolation of target molecules with minimal contamination.

2. **High Purity:** Purified molecules can be obtained in a highly pure state, essential for downstream applications.

3. **Single-Step Purification:** Often, a single affinity chromatography step can achieve significant purification, streamlining the process.

4. **Reusable Matrix:** The solid matrix can be reused after elution, making it a cost-effective approach.

5. **High Yields:** The technique typically yields a high percentage of the target molecule.

6. **Selective Removal of Contaminants:** Affinity chromatography can be used to specifically remove unwanted components, such as proteases, from a sample.

**Limitations**

1. **Time Consuming:** Compared to some other separation methods, affinity chromatography can be a time-consuming process.

2. **Solvent Costs:** Large volumes of solvents may be required, leading to potentially high costs.

3. **Labor Intensive:** The technique can be labor-intensive, particularly in terms of column preparation and optimization.

4. **Non-Specific Interactions:** While minimized, non-specific adsorption of other molecules can occur.

5. **Ligand Availability and Cost**: Immobilized ligands might be expensive and not readily available for all targets.

6. **Denaturation Risk:** Improper pH conditions during

## Gas Chromatography

Gas chromatography is distinguished from other types of chromatography by the use of a gas as the mobile phase and vapors to separate components. It is therefore used to isolate and identify low molecular weight molecules in the gas phase. The sample is either a gas or a liquid that has been vaporized in the injection port. The mobile phase for gas chromatography is a carrier gas, usually helium because to its low molecular weight and chemical inertness. The pressure is applied, and the mobile phase transports the analyte through the column. A stationary phase-coated column is used to separate the samples.

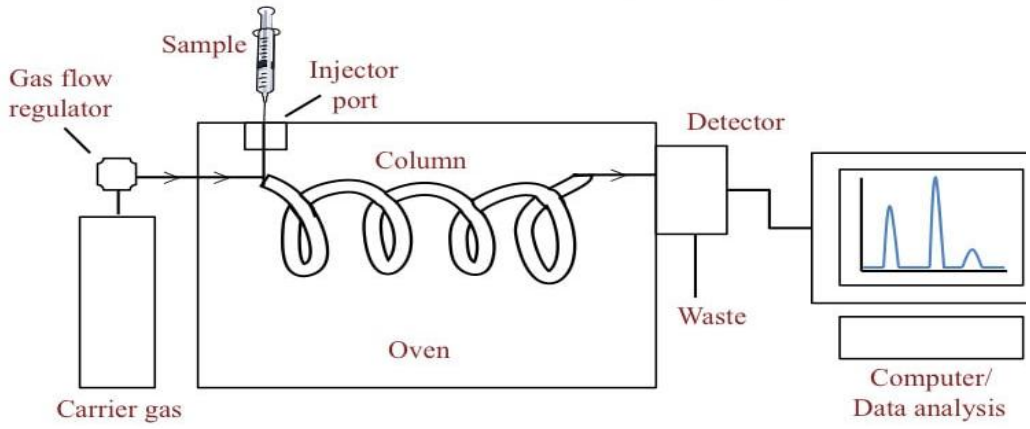


**Figure 8:** Gas Chromatography

## Principle

Gas chromatography (GC) is a separation technique used to analyze mixtures of volatile compounds. It separates components based on their partitioning behavior between two phases:

1. **Mobile Phase:** An inert carrier gas, typically helium, nitrogen, or argon, that flows continuously through the system.

2. **Stationary Phase:** A thin layer of liquid coating a solid support material packed inside a long, narrow column.

The separation relies on the differing affinities of the components in the mixture for the stationary phase. Components with a stronger affinity for the stationary phase spend more time interacting with it, leading to a longer **retention time**

(Rt) when they exit the column. Conversely, components with a weaker affinity spend less time interacting and elute from the column faster, having a shorter Rt.

## XVII. COMPONENTS OF A GAS CHROMATOGRAPH

1. **High-Pressure Cylinders:** Contain carrier gases with pressure regulators and flow meters for controlled delivery.

2. **Sample Injection System:** Introduces the sample (liquid or gas) into the heated injector port for vaporization.

3. **Separation Column:** The heart of the GC, where components are separated based on their interactions with the stationary phase.

4. **Liquid Phases:** A wide variety of liquids are used as the stationary phase, chosen based on specific separation needs.

5. **Supports:** Solid materials that provide a large surface area for the stationary phase to coat. They should be inert, thermally stable, and able to withstand packing.

6. **Detector:** Senses the eluting components and generates a signal based on their concentration or properties.

7. **Recorder:** Translates the detector signal into a chromatogram, a plot of time vs. detector response, showing peaks for each separated component.

### Procedure

- A sample is injected into the heated injector port.
- The sample vaporizes and mixes with the carrier gas.
- The mixture flows through the column, where components interact with the stationary phase.
- Components elute from the column at different times based on their retention times.
- The detector measures each component and generates a signal.
- The recorder translates the signal into a chromatogram.

### Applications

1. **Chemical Analysis:** Identifying and quantifying components in various mixtures, such as air pollutants, perfumes, forensic samples.

2. **Environmental Monitoring:** Measuring contaminants in soil, air, and water.

3. **Quality Control:** Assuring the composition and purity of products in various industries.

4. **Drug Testing:** Detecting performance-enhancing drugs in biological samples.

### Advantages

- Fast analysis times (minutes)
- High sensitivity (detects picomoles of a substance)
- Wide range of applications
- Reliable and robust for continuous operation

### Limitations

- Analytes must be volatile and thermally stable.
- Difficult to analyze large molecules (>1000 Da).
- Samples need to be free of salts and often require comparison with known standards.

## XVIII. HPLC

High-performance liquid chromatography (HPLC) is another separation technique but uses a liquid mobile phase and a solid stationary phase. It's suitable for analyzing non-volatile and thermally unstable compounds that GC cannot handle. Both techniques offer valuable tools for analyzing complex mixtures in various scientific fields.

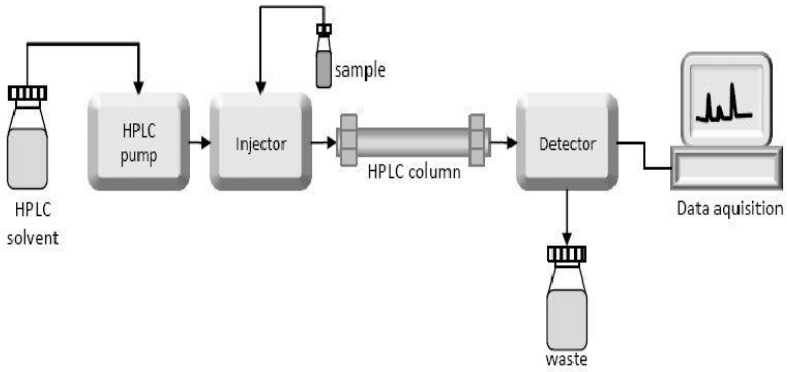


**Figure 9:** HPLC (HIGH Performance Liquid Chromatography)

## Principle of High-Performance Liquid Chromatography (HPLC)

HPLC is a separation technique that involves a stationary phase and a mobile phase. The stationary phase is a packed column containing tiny, porous particles. A liquid solvent (mobile phase) is pumped at high pressure through the column. A sample is injected into the mobile phase and carried through the column. Different components of the sample interact differently with the stationary phase, causing them to move through the column at varying speeds. As the separated components exit the column, a detector identifies them, and the results are recorded as a chromatogram.

## Supercritical Fluids

A supercritical fluid exists in a state between a liquid and a gas. It forms when a substance is heated above its critical temperature and pressure. At this point, the distinct liquid and gas phases merge.

## HPLC Instrumentation

1. **Pump:** The heart of the HPLC system, the pump delivers the mobile phase at a constant, high pressure. Modern pumps typically use reciprocating pistons.

2. **Injector:** Introduces the sample into the mobile phase flow. Manual injection using a syringe or automated injection using an autosampler are common methods.

3. **Column:** The separation takes place within the column. Stainless steel columns packed with silica or polymer gels are widely used.

4. **Detector:** Monitors the column effluent and generates a signal based on the presence of analytes. Various detector types exist, each with specific applications.

5. **Data System:** Converts detector signals into a usable format, often displayed as a chromatogram. Modern systems offer advanced data analysis capabilities.

6. **Degasser:** Removes dissolved gases from the mobile phase to improve system performance and prevent baseline noise.

7. **Column Oven:** Maintains a consistent column temperature, crucial for reproducible results and peak resolution.

## Applications of HPLC

HPLC is a versatile technique with applications across various fields:

- Pharmaceutical analysis
- Environmental monitoring
- Food and beverage quality control
- Chemical analysis
- Biotechnology
- Forensics

## Advantages of HPLC

- High speed and efficiency
- Excellent sensitivity and selectivity
- Wide range of applications

## Limitations of HPLC

- High equipment and operating costs
- Complex method development
- Limited suitability for volatile compounds (compared to gas chromatography)