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Biological Analysis Methods: Separation and Purification Methods

**Document intended for Master 1 students in Applied
Biochemistry, Fundamental Biochemistry, and
Pharmacotoxicology.**

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list of abbreviations

AC : Affinity chromatography

APIs: Active Pharmaceutical Ingredients

CDI: Capacitive Deionization

CE: Capillary Electrophoresis

DNA: Deoxyribonucleic Acid

ED: Electrodialysis

FACS: Fluorescence-Activated Cell Sorting

GC: Gas Chromatography

GMP: Good Manufacturing Practices

HIC: Hydrophobic interaction chromatography

HPLC: High-Performance Liquid Chromatography

IEF: Isoelectric Focusing

IEX : Ion exchange chromatography

K_{sp}: Solubility Product Constant

LC: Liquid Chromatography

MACS: Magnetic-Activated Cell Sorting

MTBE: Production of Methyl Tert-Butyl Ether

NF: Nanofiltration

PAGE: Polyacrylamide Gel Electrophoresis

pI: Isoelectric Point

RNA: Ribonucleic Acid

RO: Reverse Osmosis

RPC: Reversed phase chromatography

SDS-PAGE: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

SEC: Size exclusion chromatography

SFC: Supercritical Fluid Chromatography

SFE: Supercritical Fluid Extraction

SPE: Solid-Phase Extraction

TLC: Thin Layer Chromatography

UV: Ultraviolet

VBT: Valence Bond Theory

VOCs: Volatile Organic Compounds

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Preface

Preface

Various scientific disciplines, including chemistry, biology, pharmacology, chemical engineering, and the agri-food industry, rely on separation and purification techniques. These methods are essential for isolating specific components from mixtures, separating unwanted substances, or purifying products for particular applications.

These techniques are based on differences in the physical, chemical, or biological properties of the components within a mixture, such as solubility, size, density, polarity, or charge.

The separation process refers to the division of a mixture into its specific components. Physical techniques, such as filtration, distillation, or centrifugation, can be employed depending on the characteristics of the substances being processed.

The purification, on the other hand, involves removing impurities from a substance to obtain a high-purity product, which is particularly important in fields such as pharmaceuticals, food manufacturing, and the study of chemical compounds.

Separation and purification methods are widely used in both everyday applications and industrial processes. For example, in the pharmaceutical industry, it is critical to purify active ingredients to ensure their safety and effectiveness. In research laboratories, these techniques enable a deeper understanding of the chemical and biological properties of substances. They are also applied in water treatment, energy production, resource recovery, and materials manufacturing.

Mastering and optimizing different separation and purification techniques are fundamental skills for scientists and engineers, as they are critical for the quality, safety, and efficiency of final products, as well as for understanding natural phenomena.

This document is intended for Master's students specializing in Applied Biochemistry, Fundamental Biochemistry, and Pharmacology-Toxicology. It can also be beneficial for undergraduate students in biology or chemistry, as well as those preparing for doctoral school entrance exams in Biology or Chemistry.

The content covers the most commonly used separation and purification methods for isolating, characterizing, quantifying, and identifying organic synthesis products or natural products, ensuring their purity and quality.

This study material is based on my teaching in modules of Biological Analysis Techniques, as well as Biological Analysis Methods I and II, which span various specializations in biochemistry.

The courses are presented in a simplified manner and explain the concepts of separation and purification methods in organic chemistry. They are illustrated with numerous applications, including spectra, figures, and tables, to aid understanding.

This material aims to serve as a guide to better understanding the importance and applications of separation and purification methods in organic and bio-organic chemistry.

The material is divided into four chapters:

- **Chapter I:** Fundamental Principles
- **Chapter II:** Physical Separation Methods
- **Chapter III:** Chemical Separation Methods
- **Chapter IV :** Purification Methods in Biological Systems

Chapter I: Fundamental Principles of Separation and Purification Methods

I.1. Introduction

Separation and purification methods are based on the principle that the components of a mixture can be distinguished by their differing physical, chemical, or biological properties. These properties are exploited to isolate, purify, or concentrate specific components. Separation methods, therefore, can be defined as processes that alter the relative amounts of substances in a mixture. In chemical methods, this may involve starting with a completely homogeneous mixture (such as a solution) or a heterogeneous sample (e.g., a solid mixed with a liquid). Through the separation process, some particles are either partially or completely removed from the sample. Understanding these fundamental principles is essential for selecting the appropriate technique for any given task in scientific research or industrial applications.

Separation and purification processes also play a crucial role in medicine and the sciences. In the pharmaceutical industry, for instance, these techniques are essential in the production of both natural and synthetic drugs to meet health needs. In the life sciences, many advances can be directly linked to the development of new separation methods. The first step in understanding the chemical reactions of life is identifying the substances present in samples taken from biological sources.

Here are the key principles that form the foundation of separation and purification methods:

I.2. Differences in Physical Properties

The differences in physical properties refer to the various characteristics of substances that can be observed or measured without changing the substance's chemical composition. Everything we see around us is composed of matter, which is defined as anything that has mass and occupies space. Matter exists in four states—solid, liquid, gas, and plasma and each substance possesses unique properties by which it can be recognized. These properties are often used to identify materials, separate mixtures, and understand how substances interact with their environments. Physical properties, in particular, can be perceived with our senses and include features like color, density, boiling point, melting point, conductivity, hardness, and solubility. Unlike chemical properties, which involve changes to a substance's chemical structure, physical properties can be observed without transforming the material into a completely new substance. Examples of these properties include color, shape, length, and temperature-related characteristics like melting and boiling points.

Here are some key categories of physical properties and their differences:

- **Color**

Color is a visible characteristic of matter and is considered a physical property rather than a chemical one. It is perceived based on the portion of the visible electromagnetic spectrum (Figure 1) that is reflected by a substance. For example, an apple appears red because it absorbs all visible colors except red, which is reflected to the observer. Similarly, grass appears green because it reflects green wavelengths of light.

The colors we see in our daily lives correspond to wavelengths between 400-780 nm. The specific wavelengths at which different colors are observed are summarized in the table 1.

Color refers to the hue of a substance as perceived by the sense of sight, and it is influenced by factors such as light absorption, reflection, and emission. While color itself is not an inherent property of matter, its perception depends on how an object interacts with light. Humans typically perceive color through three types of cone cells in the eyes (a system known as trichromacy), which allows us to see colors within the visible spectrum. However, different species may perceive color differently; for instance, bees can detect ultraviolet light, which humans cannot.

Colors have perceived properties such as hue, saturation (colorfulness), and luminance. These properties can be mixed in two ways: additively (mixing light) or subtractively (mixing pigments). When mixed correctly, colors can create the illusion of a single wavelength of light through metamerism. Colors can also be organized into a color space, and various mathematical models are used for color reproduction in digital displays, photography, and printing.

In addition to their physical properties, colors are deeply tied to human culture and emotions. Different colors are often associated with specific feelings, activities, and even national identities. In the visual arts, color theory provides a framework for using colors harmoniously, defining relationships between primary, secondary, and tertiary colors. The study of color and its properties is known as color science, a field that also explores how colors can be reproduced and perceived across different media and contexts.

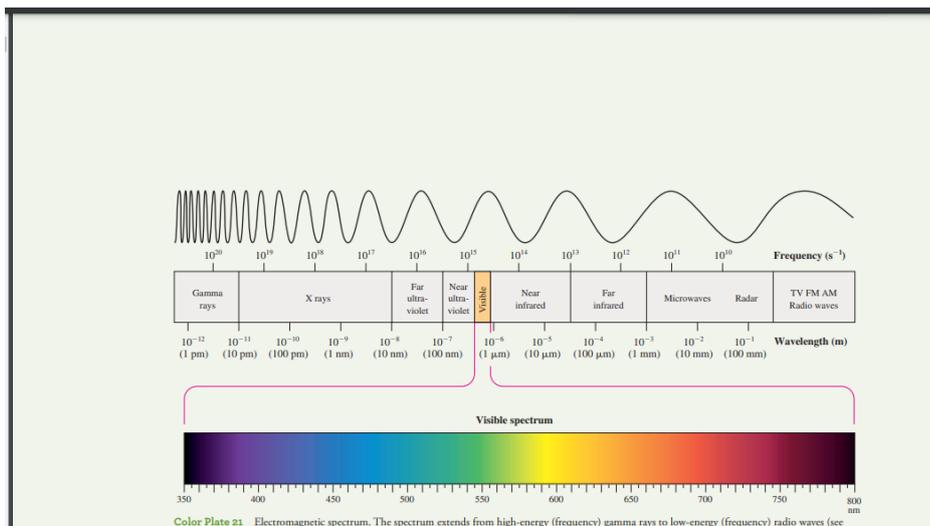


Figure 1. Electromagnetic Spectrum

Table 1. Color and Wavelength

Color	Wavelength
Violet	400-420 nm
Indigo	420-440 nm
Blue	440-490 nm
Green	490-570 nm
Yellow	570-585 nm
Orange	585-620 nm
Red	620-780 nm

- **Density and Solubility:**

Density and solubility differences are fundamental principles used in various separation techniques, such as centrifugation, sedimentation, extraction, and recrystallization.

In centrifugation, differences in density are exploited by applying a strong centrifugal force, causing denser particles to move to the bottom of the tube to form a pellet, while less dense components remain in the liquid phase (supernatant). A more specific form of centrifugation, known as density gradient centrifugation, involves creating a density gradient within the tube,

often with sucrose or other solutions, to separate particles based on their density. This technique is commonly used to isolate organelles or viruses.

Similarly, solubility plays a key role in techniques like extraction and recrystallization, where substances are separated based on their ability to dissolve in a specific solvent (figure 2). In liquid-liquid extraction, components are separated by their differing solubilities in two immiscible solvents, such as water and an organic solvent. For instance, phenol dissolves in ether but not in water, enabling its extraction into the ether phase. Recrystallization separates solid substances by dissolving them in a hot solvent and allowing them to crystallize out upon cooling, leaving impurities behind in the solution. This technique relies on the variation in solubility at different temperatures.

Mathematically, density is defined as mass divided by volume:

$$\rho = m/v,$$

where ρ represents density, m is mass, and v is volume. In some contexts, such as the U.S. oil and gas industry, density is loosely defined as weight per unit volume, though this is scientifically inaccurate. This is more specifically referred to as specific weight.

Both solubility and density differences are crucial for methods like solvent partitioning, which further separates mixtures into their components based on solubility, as seen in techniques like solid-phase extraction (SPE) and liquid-liquid extraction. Overall, density describes the mass per unit volume of a substance, while solubility refers to the substance's ability to dissolve in a particular solvent. Water, for example, has a density of 1 g/mL. These properties are essential for efficiently isolating and purifying compounds in a wide range of scientific and industrial applications.

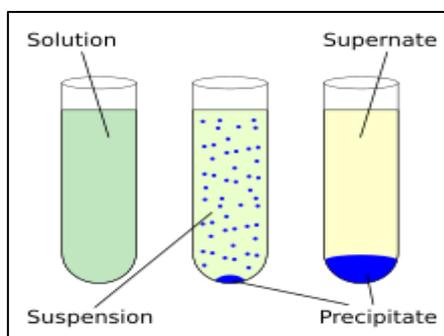


Figure 2. Example of a dissolved solid

Table 2. Some density examples of common elements and substances at 0 °C temperature and 1 atm pressure.

Material	Density (kg/m ³)
Hydrogen	0.0898
Helium	0.179
Air	1.23
Carbon dioxide	2
Lithium	535
Potassium	860
Ice	916.7
Wax	960
Sodium	970
Glass	2,500
Diamond	3,500
Stainless steel	7,800
Iron	7,870
Brass	8,530
Copper	8,940
Silver	10,500
Lead	11,340
Gold	19,320
Platinum	21,450

- **Boiling and Melting Points:**

Distillation and sublimation are separation techniques that rely on differences in boiling or melting points. In distillation, components of a liquid mixture are separated by heating the mixture, causing the substance with the lowest boiling point to vaporize first (figure 3). The vapor is then condensed back into liquid form, allowing the components to be isolated. Fractional distillation is a variation often used when the boiling points of the substances are close. For example, water boils at 100°C, ethanol at 78.37°C, and mercury at 356.7°C, showing how different substances have distinct boiling points.

Sublimation, on the other hand, is a process where a solid transitions directly into a gas without passing through the liquid phase. This method is typically used to purify compounds that sublime easily, such as iodine or naphthalene. While boiling point refers to the temperature at which a liquid turns into a gas, the melting point is the temperature at which a solid turns into a liquid. For instance, ice melts at 0°C, while metals like iron have much higher melting points,

over 1,500°C, demonstrating the wide range of temperatures at which different substances undergo phase changes.

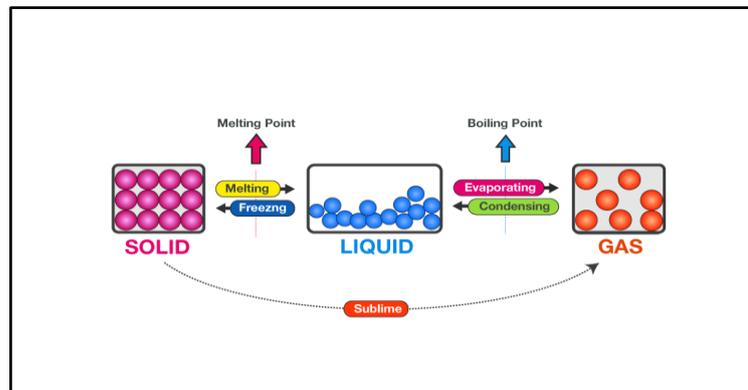


Figure 3. Melting Point and Boiling Point

- **Electrical Conductivity**

Electrical conductivity (σ) is a physical property that measures a material's ability to conduct an electric current. It describes how easily electric currents can flow through a material when subjected to an applied electric field. Specifically, it defines the relationship between the electrical current density (\vec{J}) within a material and the electric field (\vec{E}) as:

$$\vec{J} = \sigma \vec{E}$$

Conductors like copper and aluminum allow electricity to flow easily, while insulators like rubber and plastic do not. Electrical conductivity (figure 4) is the reciprocal of electrical resistivity, and it represents a material's ability to conduct electric current. The SI unit of electrical conductivity is siemens per meter (S/m). The Greek letter σ (sigma) is commonly used to denote conductivity, though κ (kappa) and γ (gamma) are sometimes used, particularly in electrical engineering. Resistivity and conductivity are intensive properties of materials, indicating the opposition of a standard cube of material to electric current, while electrical resistance and conductance are corresponding extensive properties that describe the opposition of a specific object to electric current.

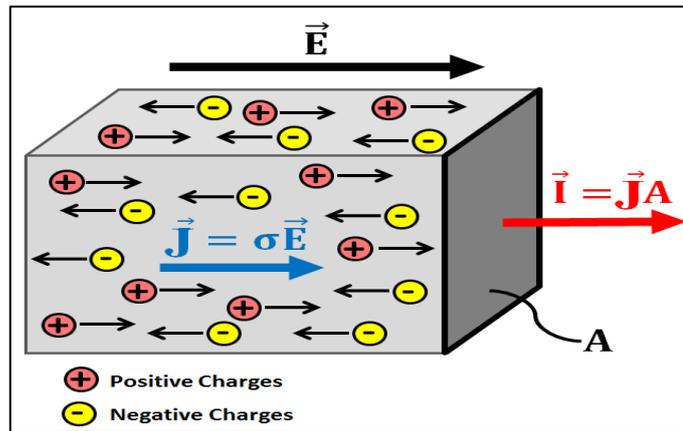


Figure 4: Conductivity in materials

- **Size**

Separation techniques that exploit differences in particle or molecular size include filtration, sieving, and centrifugation. In these methods, particles of different sizes are isolated from one another based on their ability to pass through or settle under specific conditions. For example, in filtration, larger particles or solid matter are separated from a liquid or gas phase by using a filter with pores of a specific size. Smaller molecules or ions can pass through the filter, while larger particles are trapped. Similarly, sieving is often used to separate larger solid particles, such as when separating sand from grains.

Centrifugation also takes advantage of size differences. When subjected to high-speed centrifugal force, larger particles or molecules, such as cells or organelles, will sediment at the bottom of the tube, while smaller particles or molecules remain in the liquid phase (supernatant). Another related method is membrane filtration, where a semi-permeable membrane is used to separate particles based on their size. Techniques like microfiltration, ultrafiltration, and reverse osmosis apply this principle to remove particles of varying sizes. Microfiltration removes larger particles, such as bacteria, ultrafiltration targets smaller particles like proteins, and reverse osmosis is used to remove ions and small molecules.

In summary, these techniques—filtration, sieving, centrifugation, and membrane filtration—are all effective at separating substances based on size, whether the particles are large or small, solid or dissolved.

- **Hardness**

Hardness (H) is a measure of a material's resistance to deformation, specifically its resistance to scratching. It is commonly measured using the Mohs scale of hardness, a standard scale of 10 minerals. Materials like diamond are very hard (10 on the Mohs scale), while materials like talc are very soft (1 on the Mohs scale). Hardness is determined by observing the ease or difficulty with which one mineral can scratch another or be scratched by a known substance, such as a fingernail, copper coin, steel pocketknife, or glass plate. To measure a mineral's hardness, it is scratched with a mineral or substance of known hardness, and the relative scale is used to compare it with other minerals. The mineral's hardness is determined by checking whether a substance of higher hardness can scratch it, and whether the unknown mineral can scratch a substance of lesser hardness. The Mohs scale ranks common minerals by hardness, offering a relative comparison to various materials.

- **Solubility**

Solubility refers to the ability of a substance, known as the solute, to dissolve in a solvent to form a homogeneous mixture or solution. It is a physical property, as it does not alter the chemical identity of the substances involved. Some substances dissolve easily in water, such as salt, while others do not, like oil, demonstrating differences in solubility.

In chemistry, solubility describes how a solute (typically a solid) can dissolve in a solvent (usually a liquid). Insolubility is the opposite, indicating that the solute cannot form a solution with the solvent. The extent of solubility is often quantified as the concentration of the solute in a saturated solution, where no more solute can dissolve. At this point, the system reaches solubility equilibrium. Some substances, like gases, are miscible in all proportions, meaning they can mix in any ratio, while others have a specific solubility limit.

The solute can be a solid, liquid, or gas, while the solvent is typically a solid or liquid. Both may be pure substances or solutions themselves. Gases are generally miscible in all proportions, except under extreme conditions. For a solid or liquid to dissolve in a gas, it must first transition into the gaseous state.

- **Viscosity**

Viscosity is defined as a liquid's resistance to flow or deformation when a force is applied to it. For example, honey has a high viscosity, meaning it flows slowly and resists deformation, while water has a low viscosity, meaning it flows easily and quickly. This can be observed in a simple experiment: when two cubes are dropped into two separate test tubes, the cube in the test tube containing the more viscous substance moves slowly, whereas the cube in the less viscous substance reacts more violently. One way to think about viscosity is in terms of thickness—the thicker a substance, the higher its viscosity. Thus, substances like honey and magma, which are thick and slow-moving, have higher viscosities compared to thinner liquids like water.

- **Refractive Index**

The refractive index is a measure of how much light bends or refracts as it passes through a material. It is the ratio of the speed of light in a vacuum or air to the speed of light in the material. A higher refractive index means light slows down more and bends more significantly. For example, diamond has a high refractive index, which is responsible for its characteristic sparkle, while air has a refractive index close to 1, meaning light travels through it with little bending. The behavior of light as it enters a new medium is governed by Snell's law of refraction, which states that $n_1 \sin \theta_1 = n_2 \sin \theta_2$, where n_1 and n_2 are the refractive indices of the two media, and θ_1 and θ_2 are the angles of incidence and refraction, respectively. The refractive index also affects how much light is reflected at the interface between two media, the critical angle for total internal reflection, and the intensity of reflection and refraction, as described by the Fresnel equations and Brewster's angle.

- **Thermal Conductivity**

Thermal conductivity is the ability of a substance to conduct heat, and it measures the rate at which heat energy is transferred through a material. Materials like metals, such as copper, have high thermal conductivity, meaning they transfer heat efficiently. In contrast, materials like wood or plastic are poor conductors, acting as insulators. The coefficient of thermal conductivity (λ) quantifies this transfer rate, describing how much energy flows through a unit area of the material when there is a temperature gradient across it. The rate of energy transfer depends on the number of energy carriers (such as electrons or molecules) and their movement, which is influenced by factors like pressure. As a result, the total energy flux will be higher when these carriers transport energy faster than the average speed.

- **State of Matter (at room temperature)**

A state of matter refers to the physical form in which a substance exists at a given temperature and pressure, typically as a solid, liquid, or gas. For example, water is a liquid at room temperature, oxygen is a gas, and iron is a solid. In physics, matter can exist in several distinct states, with the four most common being solid, liquid, gas, and plasma. There are also many intermediate states, such as liquid crystals, and some exist only under extreme conditions, including Bose–Einstein condensates and Fermionic condensates (at very low temperatures), neutron-degenerate matter (under extreme pressure), and quark–gluon plasma (at extremely high energies).

- **Expansion and Contraction**

Thermal expansion is the tendency of materials to change their dimensions in response to temperature changes. Most materials expand when heated and contract when cooled. For example, metals like steel expand when heated, which is why steel rails on railroads must account for temperature fluctuations. On the other hand, materials like ceramics may contract when subjected to temperature changes. Concrete, whether in a bridge, highway, or building, will also expand or contract with temperature fluctuations, regardless of the structure's size or cross-sectional area. While materials generally expand in length, area, or volume with increasing temperature, they typically contract with decreasing temperature, a phenomenon known as thermal contraction. However, there are rare exceptions, such as materials that exhibit negative thermal expansion within certain temperature ranges.

- **Magnetism**

Magnetism is the property of a material that allows it to attract or repel magnetic materials. This phenomenon arises from a magnetic field, which is generated by electric currents and the magnetic moments of elementary particles. As one of the two fundamental aspects of electromagnetism, magnetism manifests most strongly in ferromagnetic materials, such as iron, cobalt, and nickel. These materials are strongly attracted to magnetic fields and can be magnetized to become permanent magnets, which produce their own magnetic fields. Materials like wood and plastic, however, do not exhibit magnetic properties.

The intrinsic magnetic properties of materials are rooted in their atomic or molecular structure and the arrangement of electrons. The spin of the electrons, along with the number of unpaired electrons and the interactions between their spin and orbital momenta, determines the material's magnetic response at both microscopic and macroscopic levels. While only a few materials are ferromagnetic, these properties allow them to either attract or repel other magnetic objects.

- **Opacity**

Opacity refers to the extent to which a material absorbs or blocks light, or more generally, its impenetrability to electromagnetic radiation, particularly visible light. Materials like glass or water are transparent, meaning they allow light to pass through, while metals such as lead are opaque, meaning they block light entirely. This property can vary significantly depending on a material's chemical structure, atomic arrangement, and bonding, which in turn influences its potential uses in different applications.

Opacity is particularly relevant in the context of radiative transfer, where it describes the absorption and scattering of radiation within a medium, such as plasma, shielding materials, or glass. When light interacts with a substance, it can be reflected, absorbed, scattered, or transmitted. Opaque materials do not transmit any light; instead, they either absorb or reflect it entirely. This is distinct from translucent materials, which allow some light to pass through, and transparent materials, which allow light to pass through freely.

Opacity can also depend on the frequency of the light. For instance, some glass is transparent in the visible range but opaque to ultraviolet light. The absorption of light can vary further with the frequency, as seen in cold gases with absorption lines. Opacity is often quantified in various ways, and it applies to the material as a whole, affecting both the material and its components, even though the opacity value is not inherited by child elements. The concept of opacity, along with transparency and translucency, is part of a system that classifies how light interacts with different substances, including phenomena such as diffuse and specular reflection.

I.3.Differences in Chemical Properties

Chemical properties of matter refer to its potential to undergo chemical changes or reactions based on its composition, including the elements, electrons, and bonding present. These properties describe a substance's ability to react and form new substances, and they are often difficult to define without referencing "change." For example, hydrogen has the potential to ignite and explode under the right conditions, which is a chemical property. Metals, in general, react with acids, and zinc reacts with hydrochloric acid to produce hydrogen gas—another example of a chemical property.

Chemical properties can be observed through various reactions. For instance, when a compound undergoes complete combustion in oxygen, it releases energy known as the heat of combustion. Chemical stability refers to a substance's tendency to react with water or air, with reactions like hydrolysis and oxidation being key examples. Flammability is another chemical property,

determining whether a substance will burn when exposed to flame. Burning is a chemical reaction, and thus flammability is a chemical property. Additionally, the preferred oxidation state of a metal is the lowest-energy oxidation state it seeks to adopt when reacting with other elements that can accept or donate electrons.

Chemical changes occur when chemical reactions take place, but these properties cannot be directly measured simply by looking at or touching the substance. To measure chemical properties, we observe how elements interact with their environment. For example, Group 1 elements react with moist air, so they are stored in oil to prevent contact with air and water vapor. When potassium reacts with moist air, it forms potassium oxide, creating a protective layer on the metal's surface.

Here are some key categories of chemical properties and their differences:

I.3.1. Polarity

The polarity of molecules plays a central role in methods such as chromatography. Polar substances tend to interact more strongly with polar stationary phases (e.g., silica gel in thin-layer chromatography), while non-polar substances interact more with non-polar phases (e.g., hexane in reverse-phase chromatography).

In chemistry, polarity is a separation of electric charge leading to a molecule or its chemical groups having an electric dipole moment, with a negatively charged end and a positively charged end (figure 5).

Polar molecules must contain one or more polar bonds due to a difference in electronegativity between the bonded atoms. Molecules containing polar bonds have no molecular polarity if the bond dipoles cancel each other out by symmetry.

Polar molecules interact through dipole-dipole intermolecular forces and hydrogen bonds. Polarity underlies a number of physical properties including surface tension, solubility, and melting and boiling points.

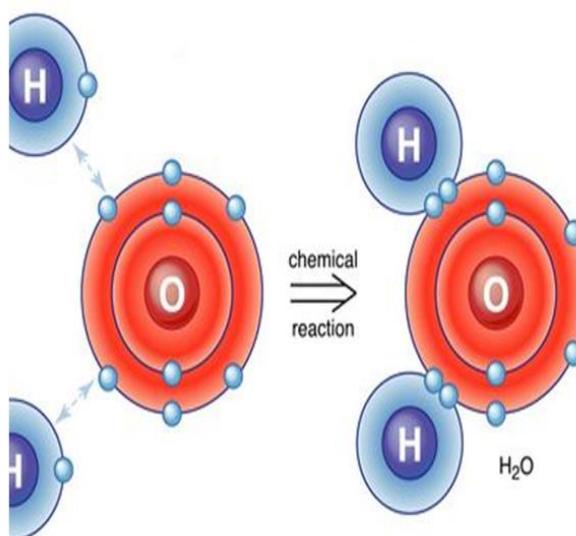


Figure 5: Polar covalent bond

I.3.2. Acidity/Basicity

Differences in pH (figure 6) can be utilized in processes like ion-exchange chromatography and acid-base extraction to separate compounds based on their charge or solubility. By adjusting the pH of the medium, you can alter the ionization state of certain compounds, making them easier to isolate. In ion-exchange chromatography, charged species are separated based on their affinity for an ion-exchange resin. Anions and cations in the mixture are exchanged with ions on the resin, and the components are eluted at different times depending on their charge and affinity. In acid-base extraction, the pH is altered to selectively protonate or deprotonate compounds, changing their solubility. Acidic compounds can be extracted into a basic solvent, and basic compounds can be extracted into an acidic solvent. These techniques rely on the chemical properties of acids and bases, which have been defined through different theoretical models in chemistry. Understanding these properties is key to exploiting pH for separation and extraction processes.

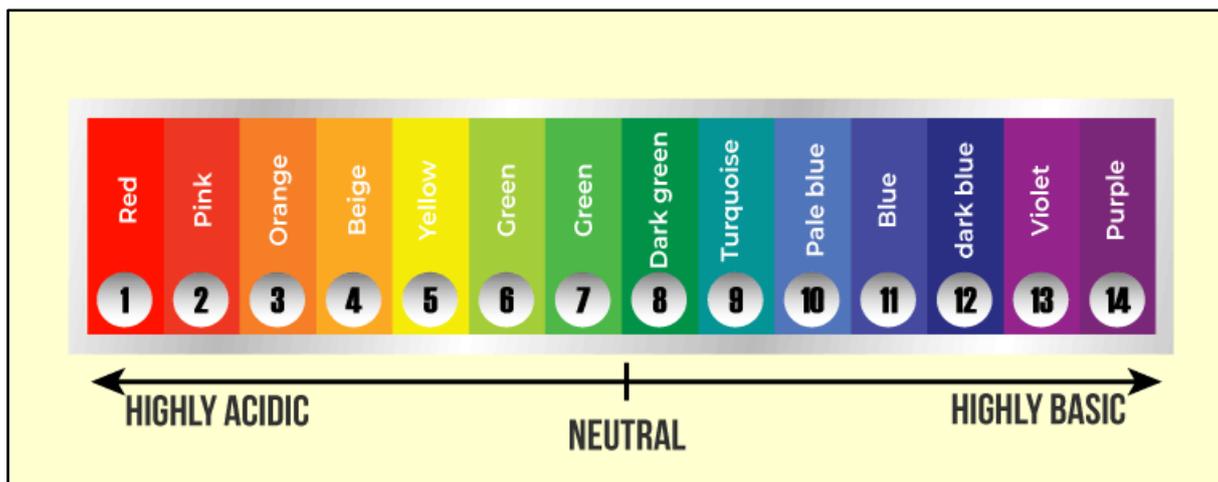


Figure 6: pH of acids and bases

I.3.3. Reactivity

Chemical reactivity can be leveraged to separate compounds. For example, selective precipitation or the use of specific reagents to form complexes with a target substance allows for its isolation from a mixture. In chemistry, reactivity refers to the tendency of a substance to undergo chemical reactions, either alone or with other materials, often accompanied by an overall release of energy.

Reactivity in chemistry involves several key aspects, including:

- The chemical reactions of individual substances,
- The interactions between two or more substances that result in chemical reactions,
- The systematic study of these reactions and their outcomes,
- Methodologies used to explore and understand the reactivity of different chemicals,
- Experimental techniques employed to observe these reactions, and
- Theories that help predict and explain these chemical processes.

When considering the reactivity of a single substance (reactant), it includes behaviors such as:

- Decomposition into simpler substances,
- The formation of new compounds through the addition of atoms from other reactants, and
- The interaction with multiple reactants to form various products.

Although chemical reactivity is complex, understanding the mechanisms of reaction intermediates and the formation of barriers can help predict reactivity, selectivity, and the pathways of reactions in both their ground and excited states. This chapter explores a mechanism based on valence bond theory (VBT), which aids in making predictions and outlining the principles that govern reactivity and selectivity in chemical processes.

I.3.4. Precipitation

Precipitation methods rely on the chemical reactivity of ions or molecules to separate compounds. When certain chemicals are introduced to a solution, they react to form an insoluble compound, known as a precipitate, which can then be isolated by filtration.

Chemical precipitation is commonly used to remove dissolved ionic metals from solutions, such as in process wastewaters containing toxic metals. In this process, ionic metals are converted into an insoluble form (solid particles) through a chemical reaction between the soluble metal compounds and a precipitating reagent.

In an aqueous solution, precipitation refers to the "sedimentation of a solid material (precipitate) from a liquid solution." The solid that forms is called the precipitate, and in inorganic reactions, the reagent causing the solid formation is known as the precipitant. The clear liquid remaining above the precipitate or the centrifuged solid phase is called the supernatant or supernate.

The concept of precipitation extends beyond aqueous solutions and can be applied in other areas of chemistry, such as organic chemistry and biochemistry. It is also relevant in fields like metallurgy, where solid impurities can segregate from a solid phase, forming a precipitate.

The notion of precipitation can also be extended to other domains of chemistry (organic chemistry and biochemistry) and even be applied to the solid phases (e.g. metallurgy and alloys) when solid impurities segregate from a solid phase

I.3.5. Complexation

Complexation is the process in which individual atoms, ions, or molecules combine to form a larger ion or molecule. In this process, one atom or ion serves as the central point of the complex, possessing empty electron orbitals that enable bonding with other atoms and unshared electrons. Specific reagents can be used to facilitate complexation, allowing for the formation of complexes with target molecules and enabling their separation from a mixture. For example, metal chelating agents are often used to isolate metal ions.

The final stage of complexation involves determining the overall charge of the complex, which is the sum of the charges of its individual components. As a result, a complex can carry a neutral, positive, or negative charge within a solution.

I.4. Differences in Biological Properties

Biological properties refer to the inherent characteristics of substances or materials that influence their interaction with living organisms or biological systems. These properties determine how a substance behaves in a biological environment, affecting its interaction with cells, tissues, or entire organisms. Understanding biological properties is crucial in various fields like medicine, pharmacology, environmental science, and biotechnology. These properties can determine whether a substance is safe, effective, or harmful in therapeutic applications, its potential for toxicity, its biodegradability, and its broader impact on ecosystems and human health. Common examples of biological properties include:

- **Toxicity:** The degree to which a substance can cause harm to living organisms.
- **Bioavailability:** The extent to which a substance can be absorbed and utilized by the body after administration.
- **Biocompatibility:** How well a material interacts with living tissues without causing adverse effects.
- **Antimicrobial activity:** The ability of a substance to inhibit or kill microorganisms.
- **Metabolism:** The process by which a substance is chemically altered by the body.

Affinity for Biomolecules

In bioseparation techniques, affinity refers to the specific and selective interaction between a biomolecule and another substance, such as an antibody, enzyme, or receptor. This principle is widely used to isolate and purify specific molecules from complex mixtures by exploiting these highly selective binding interactions. Key techniques that leverage biomolecular affinity include:

- **Affinity Chromatography:** This technique uses a column packed with a ligand that specifically binds to the target molecule (e.g., an enzyme or antibody). The target molecule is separated from other components in a mixture through this binding interaction. Once bound, the target can be eluted by altering the column conditions, such as changing the pH or ionic strength, which disrupts the affinity interaction. This method

is highly specific and efficient for isolating proteins, enzymes, antibodies, and other biomolecules based on their binding affinities.

- Immunoprecipitation: This biological technique isolates specific proteins or other biomolecules from a mixture by using antibodies that bind selectively to the target molecule. The antibody-protein complex is then precipitated out of solution, allowing the target biomolecule to be separated and analyzed.
- Electrophoretic Mobility: Electrophoresis is a technique that separates charged molecules, such as proteins or nucleic acids, based on their size and charge under the influence of an electric field. The molecules move at different rates depending on their charge-to-size ratio, making it a powerful tool for molecular analysis.

Two key types of electrophoresis are:

- ✓ Gel Electrophoresis: This method separates charged molecules, such as proteins or DNA, by applying an electric field to a gel matrix. Smaller molecules move faster through the gel, while larger molecules experience greater resistance and move slower. This technique is commonly used to analyze and purify proteins, nucleic acids, and other biomolecules.
- ✓ Isoelectric Focusing (IEF): IEF is a specialized form of electrophoresis used to separate proteins based on their isoelectric point (pI), the pH at which the protein has no net charge. When an electric field is applied, proteins migrate to the pH region where their net charge is zero. This method is particularly useful for separating complex mixtures of proteins that differ in their isoelectric points.
- Cellular Characteristics: Biological separation techniques often capitalize on the distinctive characteristics of cells or biomolecules, such as size, shape, surface markers, and density. These properties are used to sort and isolate specific cell types or other cellular components. Important techniques include:
 - ✓ Flow Cytometry: Flow cytometry uses a laser and fluorescence to measure and sort cells based on their size, granularity, and specific surface markers. Fluorescent dyes are used to label cells with specific characteristics, allowing researchers to separate different types of cells from a mixed sample. This method is widely used in immunology, cancer research, and diagnostics.
 - ✓ Cell Sorting: This involves separating cells based on physical properties such as size, shape, and surface markers. Methods like Magnetic-Activated Cell Sorting (MACS) and

Fluorescence-Activated Cell Sorting (FACS) are commonly used to isolate specific populations of cells. MACS uses magnetic beads attached to antibodies to sort cells, while FACS uses fluorescence to identify and separate cells with specific markers. These techniques are essential for cell-based research, cancer therapy, and stem cell isolation.

I.5. Thermodynamic Principles in Separation

Thermodynamics plays a crucial role in various separation processes by providing the fundamental principles (figure 7) that govern phase transitions, partitioning, and diffusion. These thermodynamic concepts are vital for understanding how substances can be isolated from mixtures based on their distinct physical and chemical properties.

Phase transitions involve changes in the state of matter, such as from solid to liquid (melting), liquid to gas (evaporation), or gas to liquid (condensation). These transitions are governed by the relationship between temperature, pressure, and the substance's specific phase transition points. Many separation techniques leverage these transitions to isolate components of a mixture. For instance, in evaporation/condensation, the separation of liquid mixtures with different boiling points is based on thermodynamic principles. In distillation, the component with the lower boiling point evaporates first, and its vapor is condensed back into a liquid. This process exploits the different boiling points of the compounds in the mixture, enabling their separation, as seen in industries like petrochemical refining and alcohol production. Similarly, sublimation involves a substance transitioning directly from a solid to a gas, bypassing the liquid phase. This is useful in purification processes, such as the purification of iodine and camphor, driven by controlled pressure and temperature changes.

Another important thermodynamic concept is the partition coefficient, which quantifies the distribution of a substance between two immiscible phases. In liquid-liquid extraction, the partition coefficient indicates how a compound is distributed between two solvents, often water and an organic solvent. The higher the partition coefficient, the greater the solubility of the compound in one phase over the other. This principle is vital in processes such as pharmaceutical extraction or environmental analysis, where compounds are separated based on their solubility. In chromatography, the partition coefficient of each component in a mixture determines its rate of movement through the stationary phase, enabling the separation of components based on their affinity for the stationary or mobile phases.

Diffusion, the movement of molecules from areas of high concentration to low concentration, is another key thermodynamic principle underlying separation techniques. In chromatography,

smaller molecules diffuse more quickly through the medium than larger ones, facilitating separation based on size and molecular properties. Similarly, dialysis utilizes differential diffusion across a semi-permeable membrane to separate molecules. This is particularly useful in medical applications, such as kidney dialysis, where small molecules like urea diffuse across the membrane while larger molecules, such as proteins, are retained.

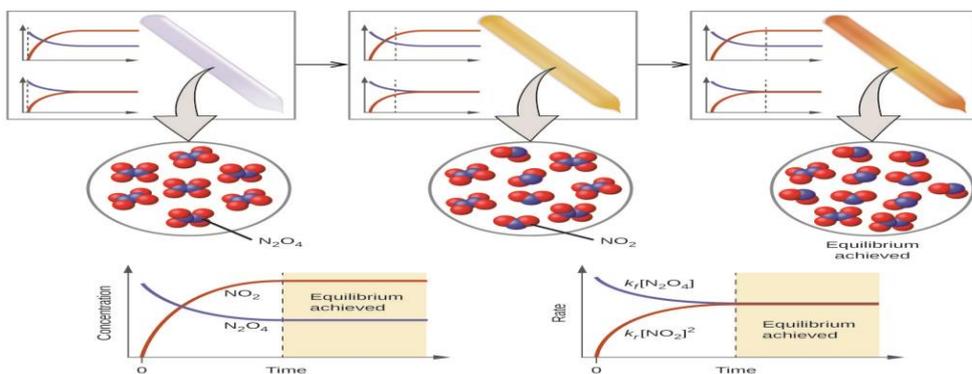


Figure 7: Chemical equilibria

I.6. Energy Considerations in Separation Processes

Energy plays a critical role in separation processes, as it is often required to overcome the intermolecular forces that hold the components of a mixture together. These forces, which can include van der Waals forces, ionic bonds, or hydrogen bonds, must be disrupted or altered to achieve separation. The amount and form of energy required vary depending on the separation technique and the properties of the components being separated. This energy input is often referred to as the work of separation, which refers to the energy needed to break these interactions and allow the components to move apart (figure 8).

For example, in centrifugation, mechanical energy is used to create a centrifugal force that separates particles based on their size, shape, and density. The work involved here is the mechanical force applied to the sample to accelerate the particles, causing denser components to move toward the bottom of the tube. In distillation, heat energy is applied to vaporize the more volatile components of a mixture, which are then condensed into separate fractions. This technique requires thermal energy to overcome the intermolecular forces holding the molecules together in the liquid phase, allowing for the separation of components based on their boiling points. In both cases, energy is essential for breaking the bonds or interactions that hold the mixture together, enabling the separation of its components.

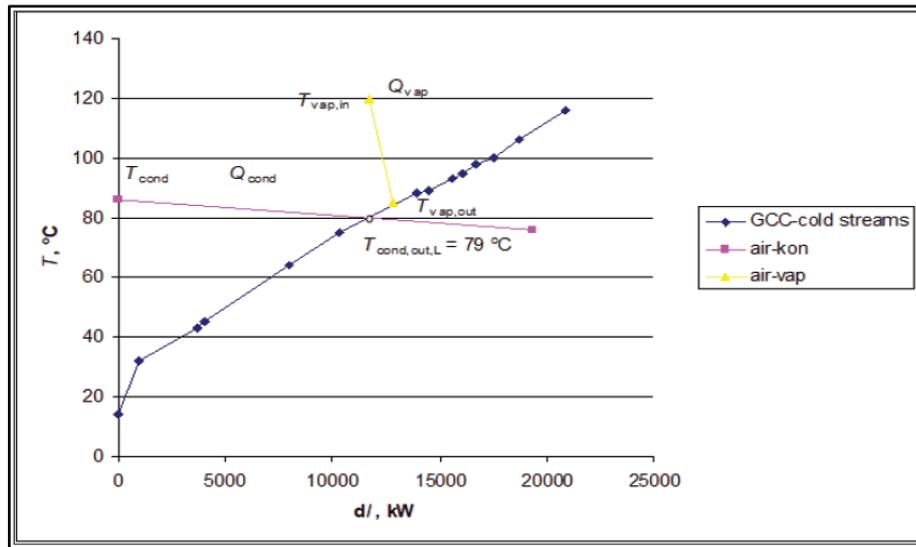


Figure 8: Energy Usage During Drying System

I.7. Kinetic Factors in Separation Processes

Kinetic factors are critical in separation processes, as they govern how fast and efficiently components in a mixture move or interact within a medium. These factors, including the rate of diffusion, the efficiency of separation, and the speed of molecular interactions, play a pivotal role in determining the success of a separation process. The rate of diffusion (figure 9), for example, is a key aspect in techniques like chromatography and dialysis, where molecules move from areas of higher concentration to lower concentration driven by concentration gradients. Several factors influence diffusion rates, including molecular size, charge, and solubility. Smaller molecules diffuse faster than larger ones due to less resistance in the medium, as observed in gel electrophoresis, where smaller molecules such as DNA fragments move more quickly than larger ones. Charged molecules also diffuse at different rates depending on their charge and the medium used, as seen in electrophoresis, where the molecules migrate faster under the influence of an electric field based on their charge. Additionally, solubility plays a crucial role, as molecules more soluble in one phase than another will diffuse at different rates, which aids in separation processes like liquid-liquid extraction. By controlling these factors, separation processes can selectively isolate components based on their diffusion rates. Furthermore, separation efficiency, which refers to how well a technique isolates distinct components, is also influenced by kinetic factors. In chromatography, for example, the balance between the time a substance spends in the stationary phase and the time it spends in the mobile phase determines separation efficiency. In high-performance liquid chromatography (HPLC), components move through a column at different speeds, depending on how strongly they

interact with the stationary phase. Similarly, in gas chromatography, the volatility and interaction of sample components with the stationary phase are key to achieving effective separation.

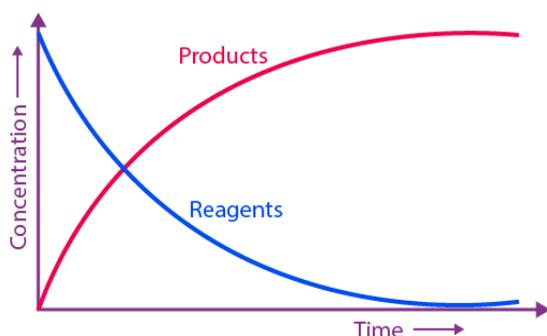


Figure 9: Rate of reaction

I.8. Selectivity and Resolution in Separation Techniques

Selective interactions and resolution are key factors that influence the effectiveness of separation methods. Selectivity refers to the ability of a technique to preferentially separate components of a mixture based on differences in their properties, such as charge, size, polarity, or affinity for certain substances. This property is crucial in methods like chromatography and electrophoresis, where even small differences in molecular properties can lead to distinct separation patterns. For instance, affinity chromatography relies on selective interactions between a target molecule and a stationary phase with a ligand or antibody attached. This specific interaction allows the isolation of a particular biomolecule from a complex mixture based on its affinity for the ligand. Similarly, electrophoresis separates molecules based on their charge and size, with molecules migrating at different rates depending on their interaction with the medium and the applied electric field.

Resolution, on the other hand, refers to the ability of a separation technique to distinguish between closely related components, ensuring that components are fully separated without overlap. Techniques like Gas Chromatography (GC) and High-Performance Liquid Chromatography (HPLC) achieve high resolution by using columns where the sample mixture interacts with a stationary phase, carried by a mobile phase. In GC, separation is

based on differences in volatility, while in HPLC, it is based on polarity. By adjusting parameters like temperature, flow rate, and column characteristics, these techniques can achieve the fine distinctions required for high-resolution separations. Capillary electrophoresis (CE) also provides high resolution through differences in the migration rates of molecules under an electric field, making it particularly useful for separating molecules with very small differences in charge or size, such as isomers or closely related proteins. Both selectivity and resolution are fundamental in achieving high-quality separations in fields like analytical chemistry, biochemistry, and pharmaceuticals, where precise separation is essential for further analysis or purification.

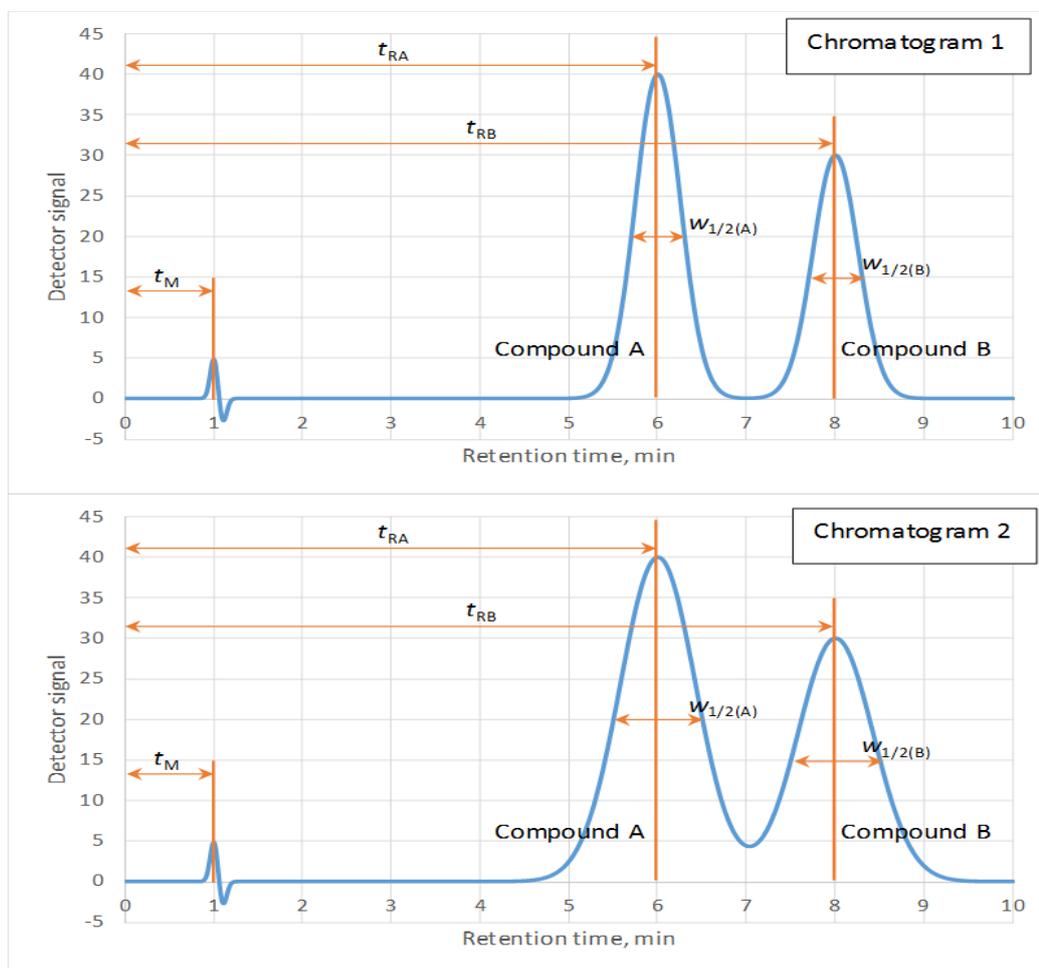


Figure 10: Chromatograms with a similar separation factor but different resolution.

Chapter II : Physical Separation and Purification Methods

II.1. Introduction

Physical separation can be defined as a set of techniques that use the distinct physical properties of substances to separate them from a mixture. These methods are based on characteristics such as size, shape, density, solubility, and the ability to respond to magnetic or electrical forces. The key feature of physical separation is that it does not alter the chemical or physical composition of the substances involved, allowing each component to retain its original properties after the process. These techniques are widely applied in industries and everyday life for tasks such as purification, sorting, and recycling.

II.2. Principles of Physical Separation

Physical separation methods leverage the distinct physical properties of substances in a mixture, such as size, state of matter (solid, liquid, gas), density, solubility, boiling point, and magnetic properties. These characteristics allow for the selection of an appropriate method to separate the components of a mixture.

For example, magnetic separation is used to separate iron filings from a mixture of sand and iron, since iron is magnetic and sand is not. Similarly, filtration is commonly employed to separate solids from liquids in heterogeneous mixtures, such as in the case of sand and water, where the filter traps the sand particles while allowing the water to pass through.

These separation techniques are based on the exploitation of the physical properties of the substances involved—such as their state of matter, density, magnetic properties, solubility, size and shape, and boiling or melting points.

The versatility and efficiency of physical separation methods make them valuable tools in isolating components from mixtures without altering their chemical composition. This makes them essential in various industries and scientific applications.

II.3. Common Physical Separation Techniques

Common physical separation techniques refer to various methods used to separate components of a mixture based on their distinct physical properties. These methods are widely used in both laboratory and industrial settings for purposes like purifying substances, recycling materials, and separating valuable components from waste. Unlike chemical processes, physical separation techniques do not alter the chemical composition of the substances being separated. Instead, they rely on the differences in physical properties of the components to achieve the desired separation. These techniques are crucial in many industries and scientific applications.

The different common physical separation techniques are:

II.3.1. Filtration

Filtration is a widely used method for separating an insoluble solid from a liquid. For instance, when separating sand from water, filtration efficiently removes the solid particles by passing the mixture through a filter, such as filter paper. The liquid that passes through is called the filtrate, while the solid that remains on the filter is known as the residue. A familiar example of filtration in everyday life is when preparing tea; a sieve is used to separate the tea leaves from the liquid, with the tea acting as the filtrate.

Filtration works by capturing particles large enough to be trapped by a porous material. The size of these particles varies depending on the mixture. For example, stream water may contain microscopic organisms like bacteria, viruses, and protozoa, which can be removed using specialized filters. In contrast, larger particles, such as soil, can be filtered using materials like coffee filters.

There are different types of filters suited for specific filtration needs:

- **Filter Paper:** A common porous material used to filter small particles in laboratories or for everyday tasks like brewing coffee.
- **Sieve:** A filter designed for larger particles, such as separating tea leaves or straining pasta.
- **Membrane Filters:** Used in laboratories or medical settings, these filters have fine pores capable of removing microorganisms from liquids.
- **Activated Carbon Filters:** These filters use charcoal to remove contaminants, commonly found in water filtration systems.
- **Sand Filters:** Often used in water treatment plants, these filters use sand layers to remove suspended solids and particles.

Each of these filter types is selected based on the size of the particles being filtered and the specific requirements of the filtration process.

In both laboratories and industries, filtration plays a critical role in ensuring the purity of substances. In labs, filter paper and membrane filters are used to precisely separate fine particles from liquids, ensuring the integrity of experiments that require purity. In industries such as pharmaceuticals and food processing, advanced filtration techniques are employed to remove impurities from raw materials or finished products, ensuring safety, quality, and compliance with regulations. Filtration is also a key process in water treatment plants, where large-scale sand and activated carbon filters are used to purify water for consumption.

II.3.1.2. Principle of Filtration

Filtration is a technique used to separate the components of a mixture when one component is in the liquid phase and the other is in the solid phase. This is achieved by using a filter, which retains solid particles that are larger than the pores (holes) of the filter. The liquid that passes through the filter is called the filtrate, while the solid collected in the filter is known as the residue.

There are three types of filtration:

- Gravimetric Filtration
- Vacuum Filtration
- Pressure Filtration

II.3.1.3. Gravimetric Filtration (Gravity Filtration)

This method involves using filters, typically made of paper, which can be cone-shaped or pleated, through which the liquid flows under the influence of its own weight. In this process, a laboratory funnel equipped with filter paper is used. The pressure difference is created by the height of the liquid above the filter.

II.3.1.3.1. Principle

This method relies on the use of a filter made of mesh that allows water to pass through while retaining the particles it contains. Filtration, therefore, results in a homogeneous liquid. The pores of filter paper are so small that they do not allow any particles larger than a bacterium to pass through. The large particles trapped on the filter paper form the residue, while the liquid that passes through the filter is called the filtrate.

II.3.1.3 .2. Setup

To perform filtration, a filter and a supporting device, called a filter holder, are required. Most often, a funnel is used for this purpose (figure 11).

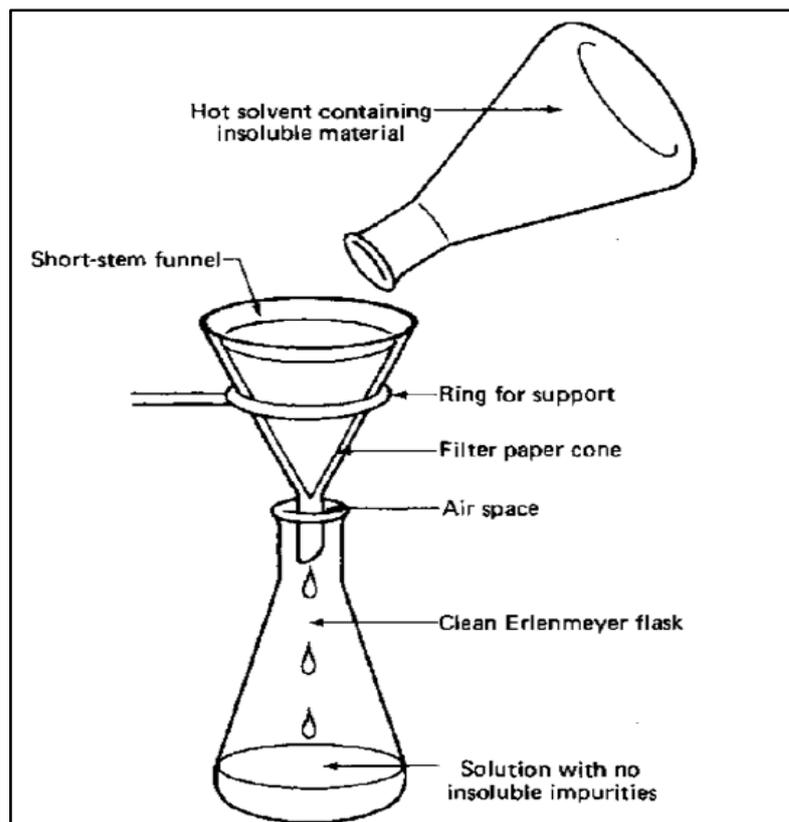


Figure 11: Gravity Filtration Setup

Drawbacks of Gravimetric Filtration:

- Filtration is slow.
- Recovering the solid phase is challenging, especially when it is in small quantities.
- The separation is incomplete: the solid retains a significant amount of liquid.

This method is generally slow and does not provide an optimal separation of the solid and liquid. To overcome these drawbacks, vacuum filtration is often employed.

II.3.1.4. Vacuum Filtration

The filtration speed is increased by creating a vacuum downstream of the filtering material (figure 12). This method is commonly used with sintered glass and filter membranes. Special Büchner funnels, attached to a vacuum flask, are used to create the vacuum. The funnel is sealed onto the flask with a rubber stopper. The funnel, made of porcelain or plastic, forms a tight seal with the flask once the vacuum is applied.

In some cases, the solid may consist of very fine particles that could pass through the filter. In such instances, a sintered glass funnel, into which the mixture is directly poured, can be used.

Various porosities of sintered glass funnels are available, and it is important to choose the one that is suitable for the particle size of the solid being filtered.

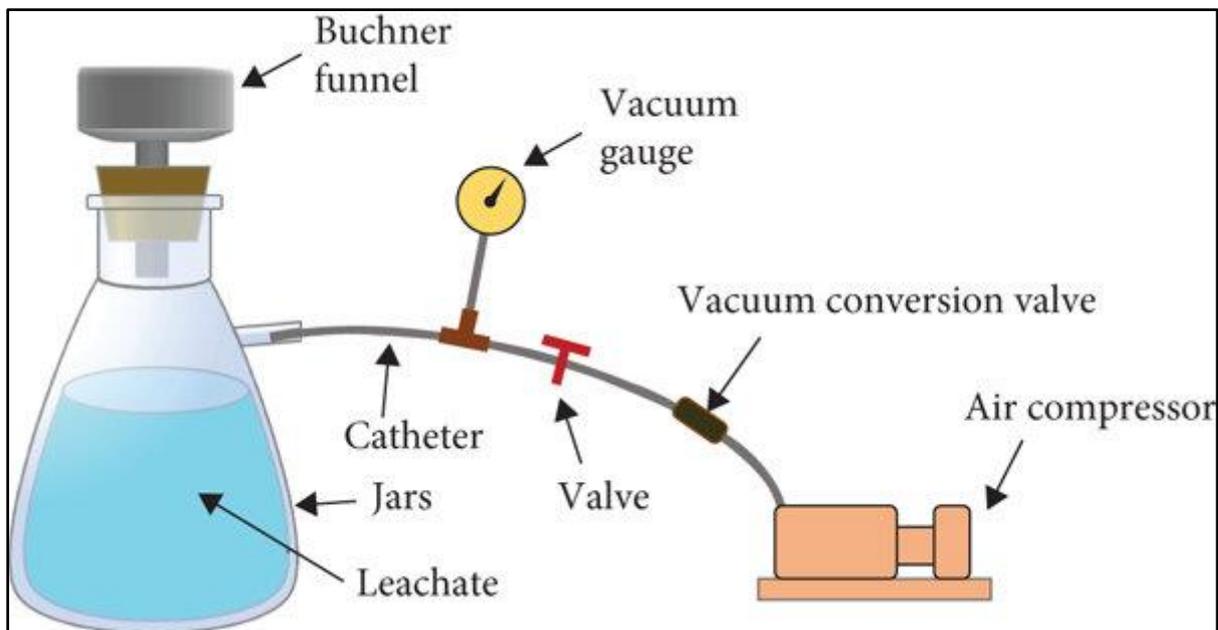


Figure 12 : Diagram of Vacuum Filtration

- **Büchner-type filter:** Made of porcelain, this cylindrical filter features a coarse mesh. A circular paper filter, large enough to cover the entire mesh, is placed on top.
- **Sintered glass filter:** This is a glass funnel containing a sintered glass disc with a fixed porosity. This type of filter is used in extreme pH conditions where paper filters would not withstand. However, this filter cannot be used with hydrofluoric acid solutions, as it reacts with the silica in the glass.

II.3.1.5. Pressure Filtration

The filtration speed is increased by applying pressure to the liquid to be filtered upstream of the filtering material, represented by a filter membrane (figure 13). Pressure filtration prevents foaming and solvent evaporation, making it commonly used in industrial applications. This pressure filtration system with filter membranes is also available in the form of filter cartridges (such as Millipore) that can be adapted to a syringe, making it convenient for filtering small volumes of solution. In the laboratory, sterilizing microfiltration using the Swinnex Millipore device is a form of pressure filtration. This device consists of two plastic pieces that are screwed together, enclosing a filter membrane.

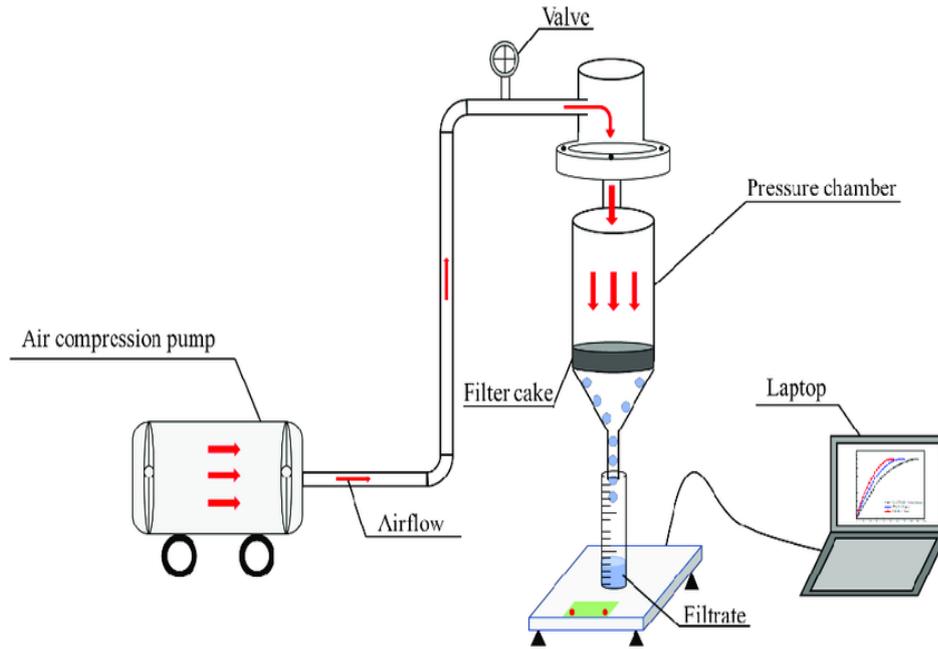


Figure 13: Pressure Filtration

II.3.1.6. Performing Filtration

- The first step is selecting an appropriate filter with a mesh size small enough to retain solid particles while not overly restricting the flow rate.
- The filter can be placed in a funnel to facilitate the collection of the filtrate.
- The heterogeneous mixture to be filtered is poured slowly and in stages to avoid damaging the filter or overwhelming it. For instance, the mixture can be poured along a glass rod to control the flow.
- The solid residue is collected in the filter. If the goal of the filtration is to obtain the residue, it can be dried in an oven.
- The filtrate can be collected in an Erlenmeyer flask.

II.3.2 Centrifugation

Centrifugation is a separation method used when filtration is ineffective, particularly when solid particles in a liquid are small enough to pass through a filter. It is ideal for separating insoluble substances suspended in liquids where particles are too fine to be captured by conventional filtration. Centrifugation relies on several factors, such as particle size, shape, and density, the viscosity of the liquid medium, and the speed of the centrifuge. The principle behind centrifugation (figure 13)is that denser particles are driven outward by centrifugal force,

settling at the bottom of the centrifuge tube, while lighter particles remain at the top. This simulated gravitational force accelerates the separation of components in the mixture. The denser components form a pellet at the bottom, and the remaining liquid, now separated from the solid particles, is called the supernatant or supernate.

Centrifugation is a mechanical process that increases the effective gravitational pull on the mixture, significantly speeding up particle separation. Larger, denser particles separate more rapidly than they would under normal gravity. This method is widely used in both industrial and laboratory settings, offering a much faster separation technique than natural settling.

There are various types of centrifuges, each suited for different applications:

1. **Fixed-angle Centrifuge:** Tubes are placed at a fixed angle to the axis of rotation, often used for separating large particles or cellular components.
2. **Vertical Centrifuge:** Tubes are oriented vertically, allowing for efficient separation based on density.
3. **Swinging-bucket Centrifuge:** The tubes swing out horizontally, enabling better separation of particles with a wide range of densities.
4. **Microcentrifuge:** A small, tabletop centrifuge used for small sample volumes, particularly in biological and chemical research.

Centrifugation has broad applications in both biology and chemistry. In biology, it is used for isolating cell components like nuclei, mitochondria, and ribosomes, as well as isolating DNA, RNA, and proteins. It is also used for separating blood components, such as plasma, serum, and red blood cells. In chemistry, centrifugation aids in purifying chemicals, separating reaction products, and isolating precipitates in chemical processes. This technique plays a vital role in speeding up processes like separating suspended particles, clarifying liquids, and isolating specific components from mixtures, making it an essential tool in scientific research, medicine, and various industrial applications.

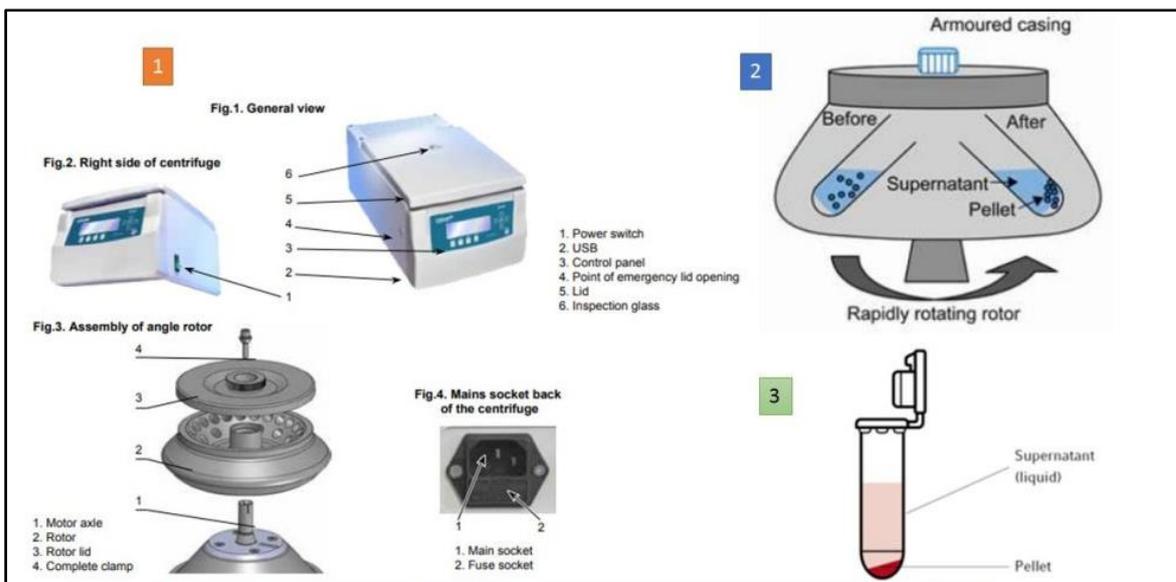


Figure 13: Centrifuge: General view, centrifugation procedure and components separation

II.3.3 Distillation

Distillation is a separation technique used to separate liquid mixtures based on differences in their boiling points. The component with the lowest boiling point vaporizes first. As the mixture is heated, the vapor passes through a cooled tube, called a condenser, where it condenses back into liquid form. This liquid, known as the distillate, is collected in a separate container. Distillation is particularly effective when the difference in boiling points between the components is at least 20°C.

There are several types of distillation techniques:

1. **Simple Distillation:** Used to separate a liquid from a non-volatile solid or when the boiling points of two liquids differ significantly. It involves a single vaporization-condensation cycle.
2. **Fractional Distillation:** Used when the boiling points of the components are close (less than 20°C difference). It employs a column with trays or packing, enabling multiple vaporization-condensation cycles for better separation.
3. **Steam Distillation:** Used to separate volatile compounds from non-volatile substances, commonly in the extraction of essential oils from plants.
4. **Vacuum Distillation:** Used for separating liquids with high boiling points by reducing pressure, which lowers the boiling points and prevents decomposition at high temperatures.

Distillation is widely used in both laboratory and industrial applications. For example, in chemical industries, fractional distillation is used to separate crude oil into products like

gasoline, diesel, and kerosene. Vacuum distillation is common in the petrochemical industry for refining heavy oils and producing high-purity chemicals. Distillation is also essential in pharmaceuticals for purifying chemicals, and in alcohol production to increase alcohol content in drinks like vodka, whiskey, and brandy.

Additionally, distillation is used in water purification to remove impurities and in the food industry for producing concentrated juices and other products. This technique is crucial for industries requiring precise separation and purification of liquid mixtures.

II.3.3.1. Principe

The principle of distillation is quite simple: a mixture of liquids is heated to reach the boiling point of one of its components. The more volatile component will evaporate first, and the vapors are collected and condensed into another container. While the first liquid evaporates (distillate), the second component does not reach its boiling point and remains in liquid form in the original container (residue). The process can be summarized in two actions :

- Heat an impure liquid or a mixture of liquids to transform them into vapors through boiling.
- Then, condense the vapors by cooling and isolate the pure liquids.

The process depends on the boiling points of the substances involved. If the boiling points are not too high ($T < 120^{\circ}\text{C}$), distillation at atmospheric pressure is sufficient. However, if the boiling points of the components are too high, a special adjustment is needed: lowering the pressure. Indeed, as pressure decreases, the boiling point (T_{eb}) of a liquid also decreases.

II.3.3.2. Setup

The setup (figure 14) starts with the heating mantle, followed by the reaction flask. Then, the distillation head, condenser, adapter, and receiving flask are added in sequence. The disassembly will be done in the reverse order.

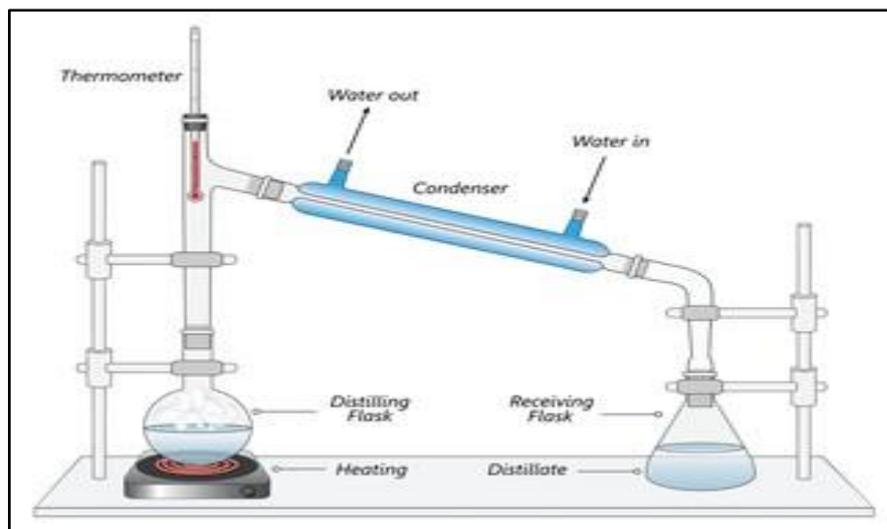


Figure 14 : Diagram of a simple distillation

II.3.3.3. Fractional Distillation

Fractional distillation is used for separating a mixture of several miscible liquids. It utilizes a separation column (or Vigreux column) to separate the different components of the mixture based on their boiling points at each plate of the column

II.3.3.3.1. Principle

Fractional distillation, also known as rectification, is a process used to separate liquids through fractionation based on differences in their boiling points. The most volatile component, with the lowest boiling point, evaporates first. The mixture is gradually brought to a boil and maintains the same temperature until the most volatile component is completely vaporized. This allows each liquid to be distilled according to its boiling point.

II.3.3.3.2. Setup

The mixture to be separated is placed in a distillation flask (boiling flask) topped with a distillation column (figure 15). At the top of the column, a vertical condenser is positioned at an incline to allow the condensed liquids to flow towards a receiving flask. A thermometer is placed at the top of the column, with its bulb positioned at the junction with the condenser, to measure the temperature of the liquid-vapor equilibrium of the component being collected in the distillate.

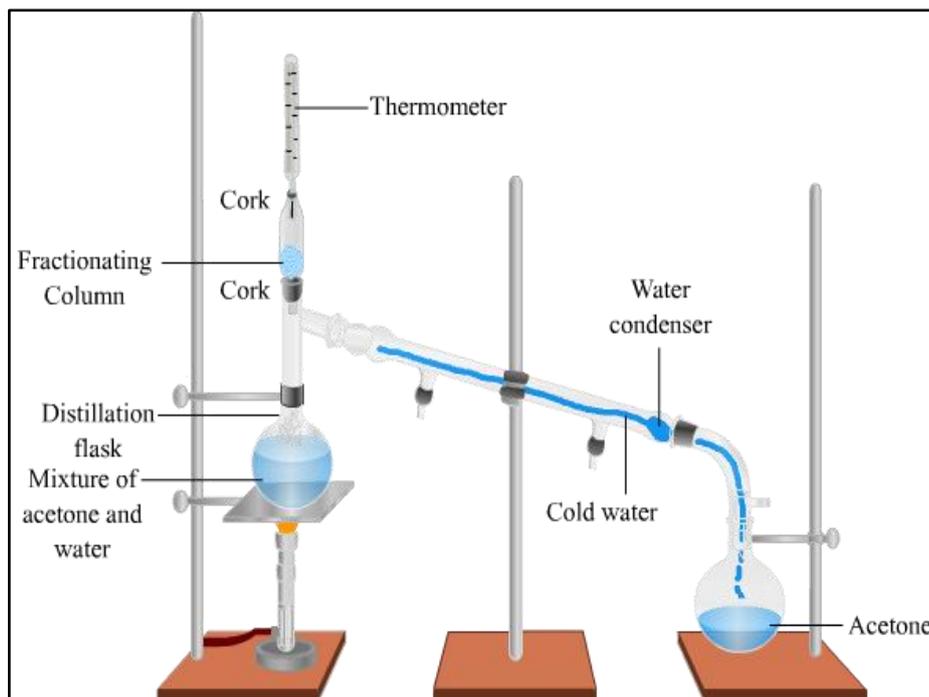


Figure 15: Diagram of a fractional distillation.

II.3.4. Chromatography

Chromatography is a widely used technique for separating the components of a mixture by passing it through a medium where each component moves at different rates. The mixture is dissolved in a fluid solvent (the mobile phase), which can be a gas or liquid, and then carried through a system like a column, capillary tube, plate, or sheet. A stationary phase, typically a solid or viscous liquid, is fixed on the surface of the system. The components of the mixture interact differently with the stationary phase, resulting in different retention times, which causes them to separate.

One of the simplest forms of chromatography is Thin-Layer Chromatography (TLC). In this method, a plate (usually made of glass or plastic) is coated with a thin layer of silica or alumina. The plate is placed in a solvent, and the solvent rises by capillary action. When a mixture is applied above the solvent level, its components move up the plate at different rates depending on how they interact with the stationary phase, leading to separation.

There are several types of chromatography, including:

1. **Gas Chromatography (GC):** Used primarily for separating and analyzing volatile compounds in gaseous form. The mixture is vaporized and passed through a column

coated with a stationary phase. The components are separated based on their different rates of movement through the column.

2. **Liquid Chromatography (LC):** Often used for non-volatile substances. The mixture is passed through a column containing a stationary phase, and components are separated based on their differential affinities for the stationary phase and the mobile phase.
3. **High-Performance Liquid Chromatography (HPLC):** A refined form of liquid chromatography that uses high pressure to push the mobile phase through the column, increasing the separation efficiency. It is widely used in analytical chemistry for separating complex mixtures.
4. **Affinity Chromatography:** This method exploits the specific binding properties between a substance and a particular ligand or receptor. It is often used in biochemistry to isolate proteins or other biomolecules from complex mixtures.

Chromatography is a fundamental technique in analytical chemistry and biochemistry. It is used to analyze and purify chemicals, identify compounds, and determine their structure. In biochemistry, chromatography plays a crucial role in purifying biomolecules such as proteins, nucleic acids, and lipids. Gas chromatography and liquid chromatography are commonly applied in both environmental and pharmaceutical analyses to identify and quantify components in complex mixtures. HPLC is particularly valuable in the pharmaceutical industry for quality control, where it ensures the purity and composition of drug products. Additionally, affinity chromatography is crucial for purifying and studying biological molecules, including enzymes, antibodies, and hormones, by leveraging their unique interactions with specific ligands.

II.3.4.1. Principle of chromatography

The fundamental principle of chromatography is to move the sample using an eluent, either gas or liquid, known as the mobile phase (MP). In contact with a second phase, immobilized on a support—whether in the form of a column or a flat surface—this mobile phase moves. The stationary phase (SP), an immobilized phase, is insoluble in the mobile phase.

A distribution coefficient, called the Nernst coefficient (K) or partition coefficient, determines the distinctive characteristics of each compound present in the sample. This coefficient, also known as the distribution ratio, defines the partitioning of a compound between the mobile and stationary phases as it moves through the chromatographic system. The chemical properties of each compound affect its interactions with both the mobile and stationary phases, resulting in different retention times and, ultimately, the separation of the sample's components.

$$K = C_s / C_m$$

C_s : concentration in the stationary phase

C_m : concentration of the solute in the mobile phase.

II.3.4.2. General chromatography terminology

- **Chromatogram**

The chromatogram is a curve that reflects the change in a parameter related to the instantaneous concentration of the solute exiting the column over time (figure 16). Time (or, rarely, elution volume) is plotted on the x-axis, and the detection signal intensity on the y-axis. The baseline represents the trace obtained in the absence of any eluted compounds. A complete separation occurs when the chromatogram shows as many chromatographic peaks returning to the baseline as there are compounds in the mixture being analyzed.

Each component is characterized by its retention time, t_R , which represents the time elapsed between the injection moment and the point on the chromatogram where the peak maximum associated with that compound occurs. Ideally, t_R is independent of the injected quantity. A non-retained component exits the column at the dead time, t_M (also called t_0).

The difference between the retention time and the dead time is called the reduced retention time, t'_R . In quantitative analysis, the primary goal is to separate the components to be quantified from the mixture. If the signal from the detector varies linearly with the concentration of a compound, the same linear relationship will apply to the area under the corresponding peak in the chromatogram.

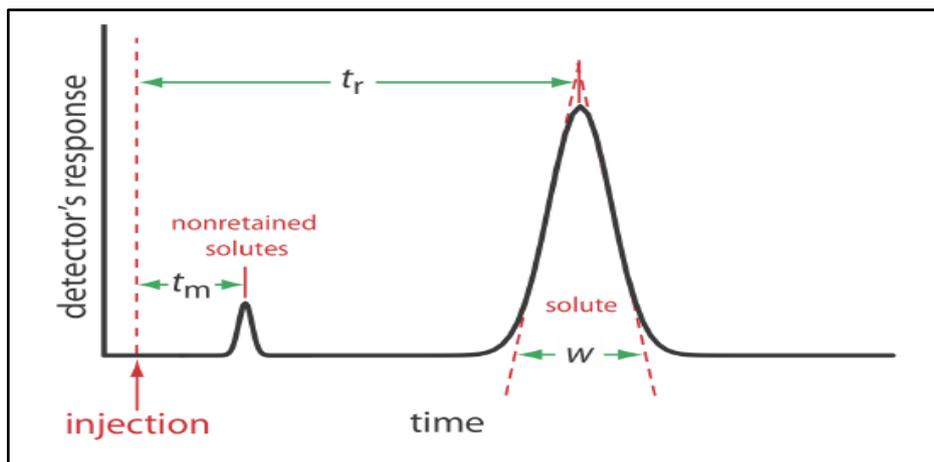


Figure 16: Chromatogram showing a solute's retention time, t_r , and baseline width, w , and the column's void time, t_m , for nonretained solutes.

- **Elution peaks**

Elution is the process of driving a solute through the stationary phase by the movement of the mobile phase. In the case of liquid-solid chromatography, the mobile phase is often referred to as the eluent.

- **Plateau model**

For half a century, various theories aimed at modeling chromatography have been proposed and continue to be developed. The most well-known include statistical approaches (stochastic theory), the plateau model, and the molecular dynamics approach.

To explain the mechanism of migration and separation of compounds within the column, the oldest model, known as Craig's plateau model, is a static approach that is considered outdated but still provides a simple description of separations.

Retention volume

Given the flow rate (D) of the mobile phase, which is assumed to be constant, the retention volume (V_r) is defined as:

$$V_r = t_r \times D$$

$$D V_r = t_r \times u \times s \times \epsilon$$

Where:

- u : average linear velocity of the mobile phase
- s : cross-sectional area of the column
- ϵ : porosity of the stationary phase (≈ 0.75 for porous silica)

The volume of the mobile phase in the column, also referred to as the dead volume, can be calculated from the chromatogram, provided an unretained solute is introduced into the system.

This is expressed as:

$$V_m = t_m \times D$$

The volume of the stationary phase (V_s) is calculated by subtracting the mobile phase volume from the total internal volume of the empty column.

- **Average Linear Velocity of the Solute and Mobile Phase**

The average linear velocity of the solute is given by:

$$v = L / t_r$$

Where:

- L : length of the column

The average linear velocity of the mobile phase, u , is given by:

$$u = L / t_m$$

Where:

t_m : dead time (time taken for a non-retained solute to pass through the column)

- **Capacity Factor**

The capacity factor (or retention factor), k' , represents the ratio of the amount of solute in the stationary phase to the amount of solute in the mobile phase. This dimensionless factor can be related to the retention time by the following expression:

$$k' = (t_r - t_m) / t_m$$

Where:

- **t_r** : retention time
- **t_m** : dead time

k' is also the ratio of the time a species spends in the stationary phase to the time it spends in the mobile phase.

Low values of k' indicate weakly retained compounds, while high values of k' indicate strongly retained compounds.

Values of k' indicate strongly retained compounds, with k' typically ranging $1 < k' < 10$ in practice.

Retention time and volume are related to the capacity factor by the following equations:

$$t_r = t_m(1 + k')$$

and

$$V_r = V_m(1 + k')$$

- **Selectivity Factor**

To describe the distance between the peaks of two compounds, the selectivity or separation factor (α) is used. This factor is defined as the ratio of the capacity factors of the two solutes that are to be separated. It is expressed as follows:

$$\alpha = (t_{r2} - t_m) / (t_{r1} - t_m) = K' 2 / K' 1$$

It is the ratio of the reduced retention times:

k_2' : capacity factor of compound 2

k_1' : capacity factor of compound 1

$\alpha = 1$ means no separation occurs, as the retention times are the same. The selectivity must be greater than 1. Two compounds cannot be separated unless they have $k' \neq 0$.

- **Column Efficiency**

The efficiency of a chromatographic column is measured, for each compound, by the number of theoretical plates (N) and the height equivalent to a theoretical plate (H). This theory originated from the search for a static model to describe the operation of a chromatographic column, similar to that of a distillation column. Rather than considering the continuous movement of the mobile phase, it is assumed that the phase progresses in successive steps, reaching an equilibrium with the stationary phase between each transfer. This approach allows the column to be divided into several fictive zones, called theoretical plates, where equilibria are established.

This allows the column to be fictively divided into several zones, called theoretical plates, where equilibria are established.

$$N = 16 (tr / l)$$

l: width of the peak at the base

$$N = 5.54 (tr / \omega)^2$$

ω : width of the peak at half-height

To compare columns of different lengths, the height equivalent to a theoretical plate is defined as:

$$N = L / H$$

Where **H** is the distance over which the chromatographic equilibrium is reached.

In HPLC, the height equivalent to a theoretical plate (HEPT) typically ranges between 0.001 and 1 mm.

The efficiency of chromatographic columns is related to the number of theoretical plates and the height equivalent to a theoretical plate. The retention time increases with the number of

theoretical plates or the height equivalent to a theoretical plate, for a constant column length, leading to a decrease in column size.

External factors can also influence column efficiency. Two columns with the same efficiency will have a similar L / dp ratio.

- **Resolution**

The resolution of two neighboring peaks is defined in terms of the distance between the maxima of the two peaks and their arithmetic average. Two characteristics determine the degree of overlap between the peaks:

- The distance between the peaks, measured as $tr_2 - tr_1$
- The width of the peaks at the base, l

$$R_s = 2 (tr_2 - tr_1) / (l_2 + l_1)$$

The higher the resolution, the better the separation. Two peaks are considered well-resolved if $R_s \geq 1.5$ (figure 17). For values of R_s (the ratio of the distances between the peaks) much greater than 1, the separation of the peaks is not necessarily better, but the separation time becomes unnecessarily long. Resolution, which measures the quality of a separation, can be optimized by relating the selectivity and capacity factors to the resolution. Assuming $l_1 = l_2$, combining the expression leads to Purnell's relation:

$$R_s = \frac{1}{4} \times \frac{(a - 1)}{a} \times \frac{k_2'}{k_2' + 1} \times \sqrt{N_2}$$

Separation can be improved by varying at least one of the three factors mentioned above.

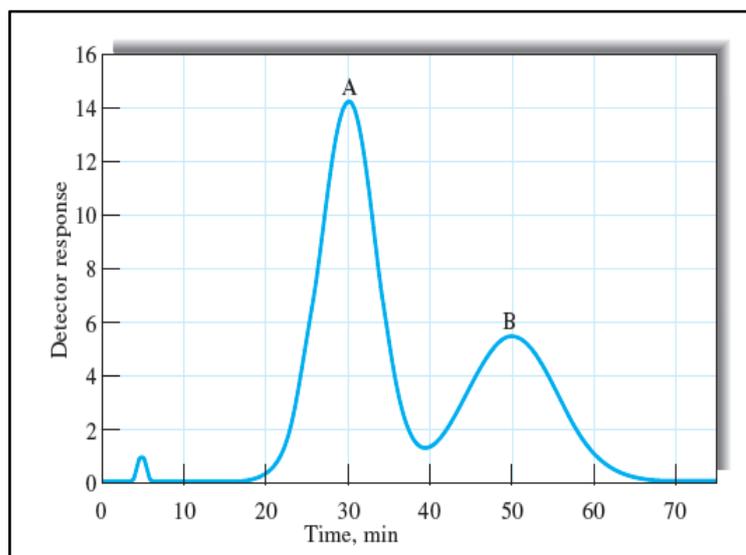


Figure 17: Chromatogram showing the separation of two components.

- **Van Deemter equation**

The equation below is the one proposed by Van Deemter et al. (1956) for gas chromatography.

$$H=A+B/u+C.u$$

The curve representing the Van Deemter equation (figure 18) is therefore a hyperbola, where A, B, and C are constants.

- **u**: average velocity of the mobile phase (carrier gas).
- **A**: represents the influence of turbulent diffusion due to flow heterogeneities.
- **B**: is a factor for molecular diffusion in the mobile phase.
- **C**: depends on the resistance to mass transfer in the liquid phase.

The plot of the HEPT curve as a function of the average velocity of the carrier gas allows the determination of the minimum flow rate that provides the best column efficiency.

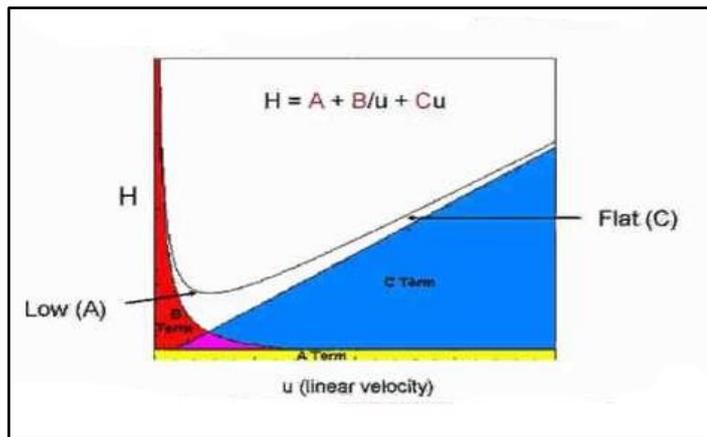


Figure 18 : Van Deemter curve.

At low velocities, the term B/u dominates, causing a negative slope and a loss of efficiency at very low speeds. At high velocities, $C.u$ prevails, leading to a positive slope and efficiency loss at high speeds. In the intermediate region, the term **A** becomes dominant, and the height equivalent to a theoretical plate (**H**) reaches a minimum. This indicates that **H** varies with **u** and achieves its minimum value when the first derivative of the function is zero.

II.3.4.3. Classification of Chromatographic Techniques

Chromatographic methods encompass a wide range of techniques that can be classified in three different ways:

- **Classification Based on the Physical Nature of the Phases:**

Depending on the physical state of the mobile phase, we distinguish the following:

1. Gas Chromatography (GC): The mobile phase is a gas, known as the carrier gas. Solutes are introduced into the column either directly if the sample is gaseous or after volatilization in the heated injection chamber if the sample is liquid (potentially after dilution or dissolution in an appropriate solvent).
2. Liquid Chromatography (LC): The mobile phase consists of a pure solvent or, more commonly, a mixture of high-purity solvents. It is introduced onto the column at a constant flow rate via a pumping system.
3. Supercritical Fluid Chromatography (SFC): The mobile phase is a supercritical fluid, which is a substance maintained beyond its critical pressure and temperature. At this point, there is no clear boundary between the liquid and gas phases.

Depending on the Physical State of the Stationary Phase, the following are distinguished:

1. Liquid/Solid Chromatography (LSC)
2. Liquid/Liquid Chromatography (LLC)
3. Gas/Solid Chromatography (GSC)
4. Gas/Liquid Chromatography (GLC)

- **Classification Based on the Phenomenon Involved:**

This classification is based on the nature of the stationary phase and its interaction with the molecules to be separated. The following types are distinguished:

1. Adsorption Chromatography
2. Partition Chromatography
3. Ion Exchange Chromatography
4. Exclusion Chromatography

5. Affinity Chromatography

• **Classification Based on the Technique Used:**

Depending on the support used in chromatography, the following types are distinguished:

1. Column Chromatography
2. Planar Chromatography
3. Paper Chromatography or Thin Layer Chromatography (TLC)

The following figure shows different types of chromatography techniques based on the type of stationary and mobile phases.

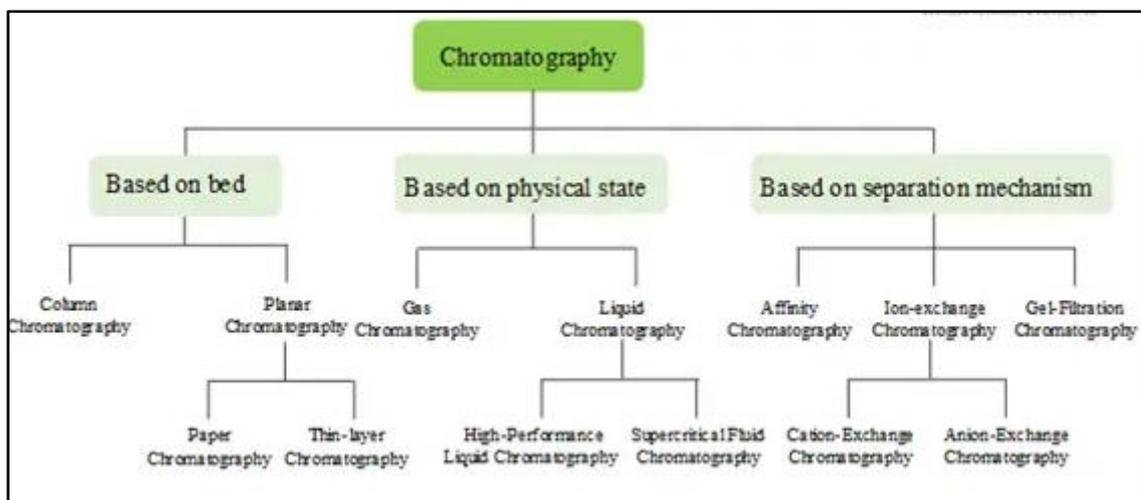


Figure 19 : different types of chromatography techniques based on the type of stationary and mobile phase

II.3.5 Magnetic Separation

Magnetic separation is a technique based on the principle that certain substances in a mixture possess magnetic properties, allowing them to be separated using magnets. This method works by exploiting the magnetic characteristics of materials such as iron, cobalt, and nickel, which are attracted to a magnetic field, while non-magnetic substances remain unaffected.

The separation is achieved through the use of either permanent magnets or electromagnets. Permanent magnets generate a constant magnetic field, while electromagnets, which create a magnetic field when an electric current is passed through them, offer adjustable magnetic

strength. These methods enable the effective separation of magnetic components from non-magnetic ones.

Magnetic separation (figure 20) is widely applied in various industries, such as recycling, where it is used to remove metals from waste materials. It is also used in the mining industry to separate magnetic ores from non-magnetic waste, in food processing to eliminate metallic contaminants, and in laboratories to isolate magnetic particles from mixtures or solutions.

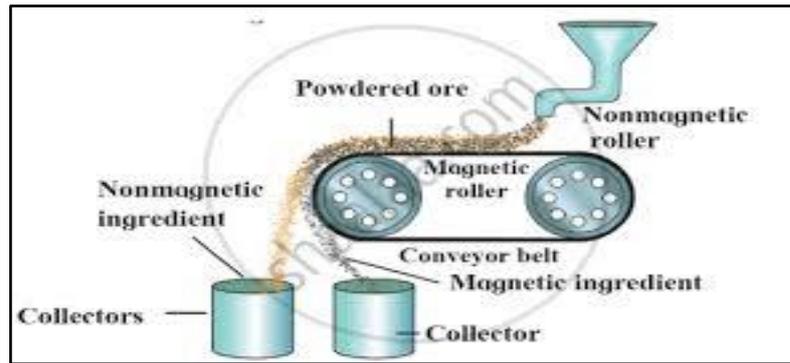


Figure 20 : Magnetic separation diagram

II.3.6. Decantation and Sedimentation

Decantation is a separation process based on the principle of gravity, used to isolate immiscible substances with different densities (mass per unit volume). In this process, denser substances settle at the bottom of a container, while lighter substances rise to the top, allowing the phases to be clearly separated. When separating liquids, for example, the mixture is left to rest in a container, such as a separating funnel. The liquid with the higher density sinks to the bottom, while the less dense liquid floats on top. Once the two phases are clearly distinct, they can be separated. A common example is the separation of water and oil, where oil floats on water due to its lower density.

Decantation can also be used to separate solid particles suspended in a liquid, a process known as sedimentation (figure 21). In sedimentation, heavier particles, such as sand, settle at the bottom of the container under the influence of gravity, while the liquid remains above. The process takes some time, allowing the particles to settle completely. Once the particles have settled, the liquid can be carefully poured off, leaving the sediment behind, typically with the help of a glass rod or similar equipment.

Decantation has significant applications in various fields, particularly in water treatment and mineral processing. In water treatment, it is used to remove solid impurities, such as dirt, silt,

and other suspended particles, in the initial stages of water purification. This helps improve the efficiency of subsequent treatment processes, such as filtration or chemical treatment, by first removing larger particles. In mineral processing, decantation is used to separate valuable minerals from gangue (waste material). For example, after ore is crushed and mixed with water, heavier minerals, such as gold or copper, settle at the bottom, while lighter materials, like waste rock, remain on top. This gravity-based separation process enables the efficient extraction of valuable minerals, making it a crucial technique in both water treatment and mining industries.

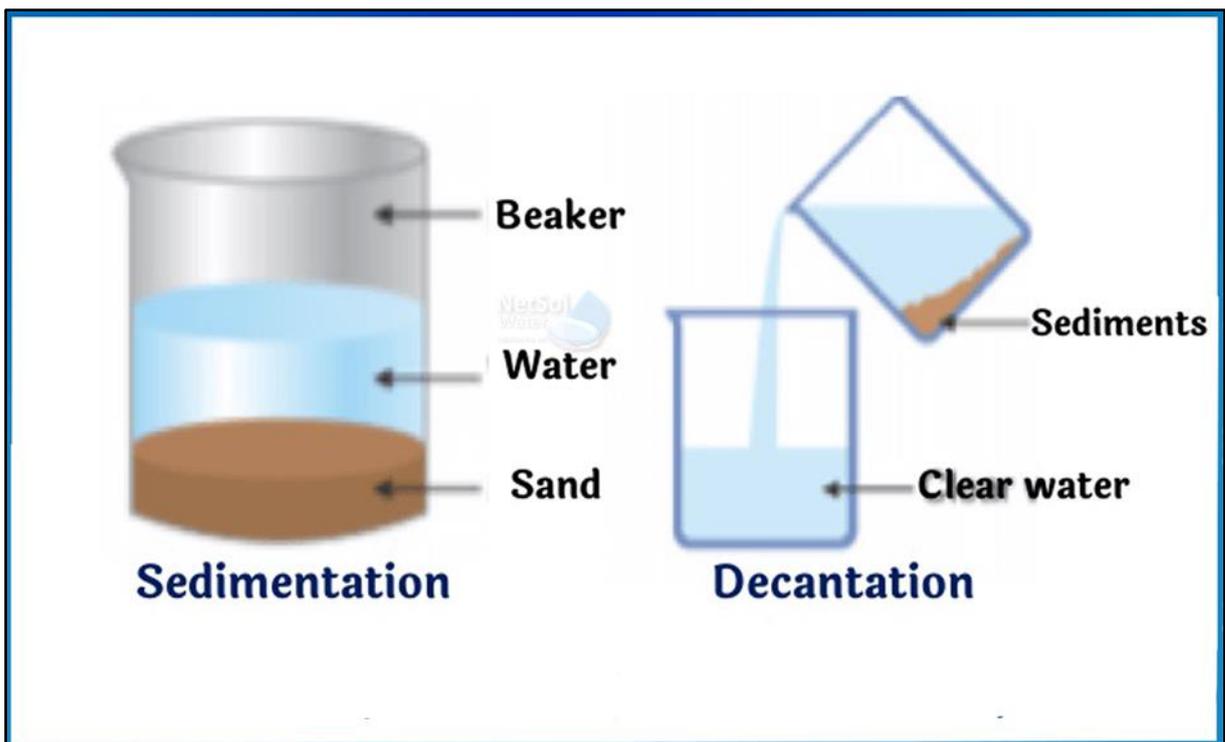


Figure 21: Difference between sedimentation and decantation

II.3.7 Evaporation

Evaporation is a technique (figure 22) used to separate a soluble solid from a liquid, typically in a solution of a solvent and a dissolved solid. The principle behind this method is the separation of the solvent from the solute through the application of heat. When the mixture is heated, the solvent, which has a lower boiling point, evaporates, turning into gas, while the solute, being non-volatile, remains behind as a solid residue. This process is driven by the absorption of thermal energy, which reduces the temperature of the substances in contact or slows down their heating by an external source. The evaporation continues until all the solvent has turned into vapor, leaving only the solid solute.

Common uses of evaporation include desalination, where water is evaporated to remove salts and other impurities, and the concentration of solutions, such as in the food and chemical industries, where solvents are removed to concentrate valuable components. Evaporation is also widely used in the production of salt from seawater and in the drying of various materials.

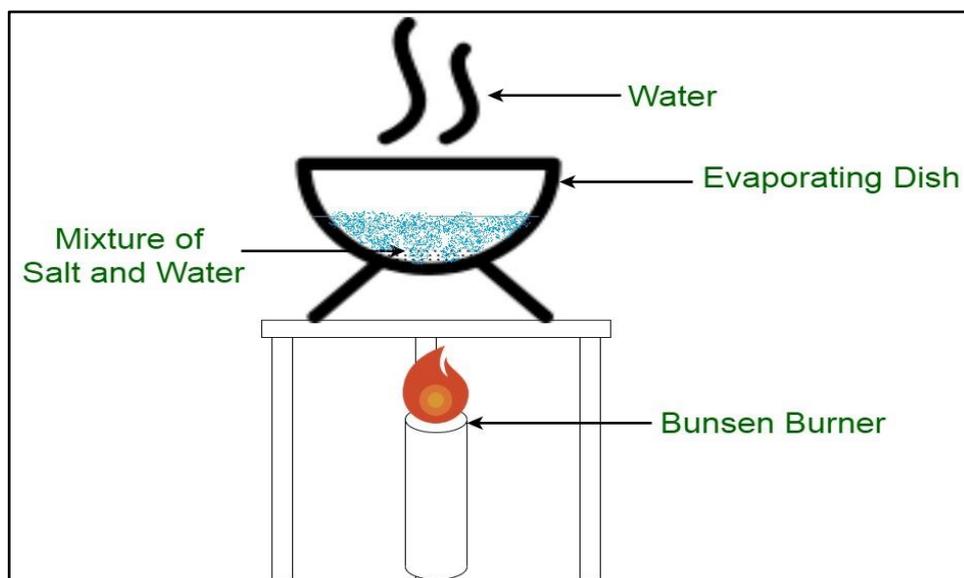


Figure 22 : Separation by evaporation

II.4. Advanced Separation Techniques

Advanced separation techniques are innovative methods used to separate complex mixtures in various industries, where traditional techniques may not be efficient or feasible. These techniques often involve sophisticated technology and principles beyond simple filtration or distillation. Examples include membrane filtration, where semi-permeable membranes are used to separate particles or molecules based on size or charge, and chromatography, which separates components based on their interaction with a stationary phase and a mobile phase. Another example is centrifugation, where high-speed spinning creates centrifugal force to separate substances of different densities. Advanced separation techniques are crucial in fields such as pharmaceuticals, biotechnology, environmental science, and materials engineering, where precision and high efficiency are required to isolate specific compounds or purify complex mixtures. These methods are essential for developing new products, improving manufacturing processes, and ensuring environmental sustainability.

II.4.1 Membrane Separation

Membrane separation (figure 23) processes use synthetic membranes as selective barriers to separate fluids. These membranes allow the relatively free passage of one component while retaining another.

The thickness of a membrane can range from 100 nm to just over 1 cm. Depending on the type of membrane, it can selectively retain or allow the passage of certain substances, whether dissolved or not, between the two phases it separates. The portion of the mixture retained by the membrane is called the retentate (or concentrate), while the portion that passes through is called the permeate. The separation occurs under the influence of a driving force, following a defined separation mechanism.

There are several types of membrane separation processes, each suited to different applications. Reverse osmosis is used to remove dissolved salts, ions, and other impurities from water by applying pressure to force the solvent through a semi-permeable membrane.

Ultrafiltration is employed to separate larger molecules, such as proteins, suspended solids, and microorganisms, from liquids, using membranes with larger pore sizes.

Nanofiltration lies between reverse osmosis and ultrafiltration, typically used for softening water by removing divalent ions, while microfiltration is used for separating larger particles like bacteria and suspended solids from liquids.

In membrane contactors, the membrane provides an interface between two phases but does not control the rate at which permeants pass through it. The characteristics of the membrane—permeability and selectivity—are crucial in determining its effectiveness in separating specific components of a mixture.

Membrane separation methods are widely applied in water purification, particularly for desalination and the removal of contaminants, helping provide clean drinking water. In biotechnology, these methods are used for processes like protein purification, dialysis, and the concentration of biomolecules. Membrane filtration is also essential in the food and pharmaceutical industries for liquid filtration and the concentration of valuable substances.

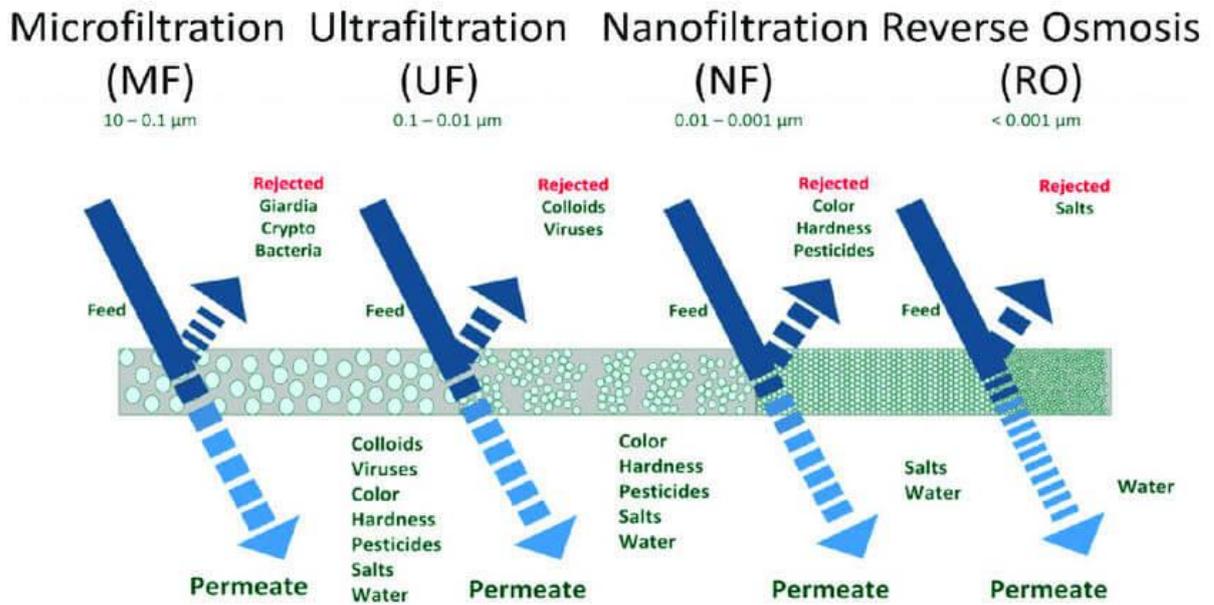


Figure 23 : Membrane separation processes

II.4.2. Electrophoresis

Electrophoresis is a laboratory technique used to separate charged molecules, such as proteins, DNA, or RNA, based on their size, charge, and shape. The process works by applying an electric field to a medium, typically a gel, which causes charged molecules to move towards the electrode with the opposite charge. Smaller molecules migrate faster than larger ones, and the rate of migration is influenced by factors such as the size and charge of the molecules. This allows for effective separation of different molecules.

Widely used in biochemistry and molecular biology, electrophoresis plays a crucial role in DNA analysis, where it separates DNA fragments by size, aiding in gene sequencing, genetic fingerprinting, and the analysis of genetic markers. In protein analysis, electrophoresis helps to separate proteins based on their size and charge, providing valuable insights into their structure, function, and interactions. It is also essential for RNA analysis and is commonly used for the purification and identification of biomolecules, making it an important tool in both research and clinical diagnostics.

The technique involves the movement of charged particles or dissolved molecules in response to an applied electric field, often involving zwitterions. For proteins, which are typically negatively charged, they move toward the positively charged anode. Electrophoresis of positively charged particles (cations) is referred to as cataphoresis, while the electrophoresis of negatively charged particles (anions) is called anaphoresis.

Electrophoresis is fundamental to various analytical techniques in biochemistry, enabling the separation of molecules or ions by size, charge, or binding affinity, either freely or through a supportive medium, using a one-directional flow of electrical charge. It is extensively used in DNA, RNA, and protein analysis for research, diagnostics, and purification processes.

II.4.2.1. Types of Electrophoresis

Electrophoresis (figure 24) can be carried out under two conditions:

- non-denaturing:

Molecules are separated in their native state, as close as possible to their natural conformation. The migration speed depends on the molecule's native charge and its three-dimensional structure.

- Denaturing Conditions:

Molecules are treated with denaturing agents before electrophoretic separation, which destroys their native three-dimensional structure. Separation occurs based on molecular mass. Denaturing agents include SDS (Sodium Dodecyl Sulfate) and β -mercaptoethanol, which reduces disulfide bonds in proteins. SDS is a mild denaturant and surfactant that acts on proteins in several ways:

- If the protein is oligomeric, its subunits are separated.
- It binds to proteins, coating them with a negative charge. Transformed into monopolyanions, the proteins all have the same electrophoretic mobility. The overall negative charge causes migration toward the anode, but the separation is based solely on molecular mass.

Depending on the electrophoretic support, there are two types of electrophoresis:

1. Free-Solution Electrophoresis (or Capillary Electrophoresis)
2. Zone Electrophoresis on a Support Medium

The following figure shows an ordinary apparatus used for gel electrophoresis:

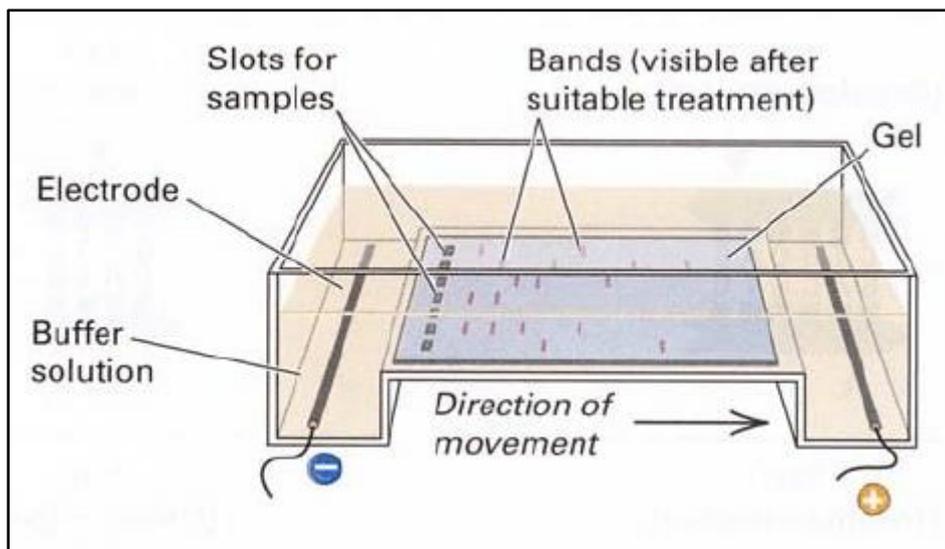


Figure 24: Ordinary apparatus used for gel electrophoresis

II.4.3 X-Ray and Neutron Scattering

X-ray and neutron scattering (figure 25) are essential techniques in material science and structural analysis used to study the atomic and molecular structure of materials. Both methods involve directing beams of X-rays or neutrons onto a sample and analyzing how the waves are scattered by the material's atoms. In X-ray scattering, high-energy X-rays interact with the electron cloud around atoms, providing detailed information about the electron density and atomic arrangement, which is particularly useful for studying crystalline materials and determining their crystal structures. In contrast, neutron scattering involves neutrons interacting with the nuclei of atoms, making it particularly valuable for studying light elements like hydrogen, which are difficult to detect with X-rays. Neutron scattering is also effective for investigating the dynamics of materials, including molecular motions and phase transitions.

Both techniques are widely applied in material science to characterize the properties of materials such as metals, polymers, and nanomaterials. They play a crucial role in structural analysis, helping to determine the arrangement of atoms in solids, investigate material behavior under different conditions, and provide insights into the molecular-level structure of complex systems. These techniques are fundamental in fields like crystallography, solid-state physics, and the development of advanced materials for electronics, energy storage, and biotechnology.

Neutron diffraction, or elastic neutron scattering, is a specific application of neutron scattering used to determine the atomic and magnetic structure of materials. A sample is placed in a beam

of thermal or cold neutrons to obtain a diffraction pattern, which reveals the material's structure. This technique is similar to X-ray diffraction, but due to their different scattering properties, neutrons and X-rays provide complementary information. X-rays are suited for superficial analysis and are ideal for studying shallow depths or thin specimens, while neutrons, with their high penetration depth, are better suited for analyzing bulk samples.

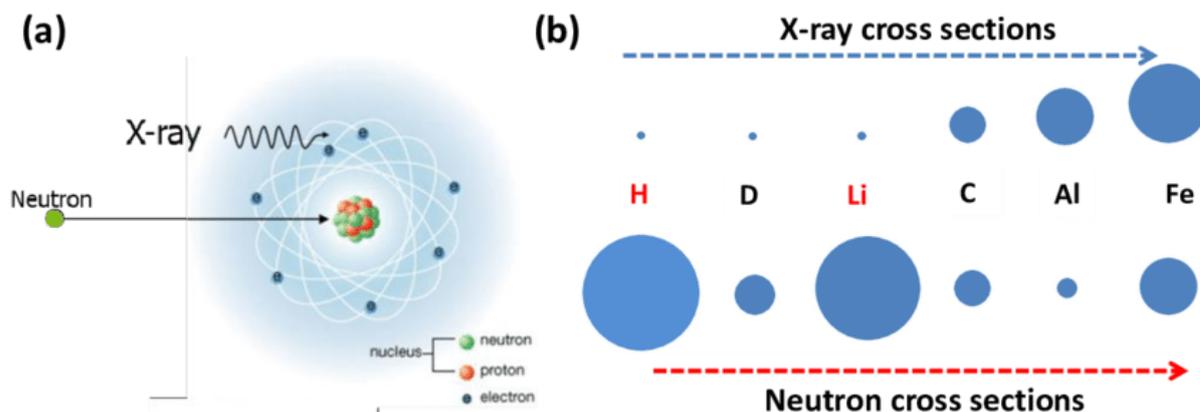


Figure 25: (a) Schematic representation of X-ray and (b) neutron cross sections

II.5. Applications of Physical Separation Methods

Applications of Physical Separation Methods are widespread across various industries, offering efficient ways to separate components based on their physical properties. These methods are commonly used in material science, mineral processing, water treatment, and biotechnology, among others. In mineral processing, techniques like gravity separation, magnetic separation, and flotation are used to extract valuable metals from ores, separating them from unwanted minerals. In water treatment, physical separation methods such as filtration, sedimentation, and membrane processes are crucial for purifying water by removing solid particles, contaminants, and impurities. These methods are also employed in biotechnology for the isolation and purification of proteins, enzymes, and other biomolecules. Additionally, physical separation techniques like centrifugation and chromatography are frequently used in pharmaceuticals to separate compounds based on size, charge, or affinity, aiding in drug development and analysis. Overall, physical separation methods are essential for ensuring the purity, quality, and efficiency of processes across a wide range of applications.

II.5.1 Industrial Applications

Industrial Applications of Physical Separation Methods play a critical role in enhancing efficiency and product quality across various industries, including mining, chemical, food, and pharmaceutical sectors. In the mining industry, physical separation techniques such as gravity separation, magnetic separation, and flotation are used to extract valuable minerals and metals from ores, while eliminating impurities and improving the quality of the final product. These methods enable the processing of raw materials like gold, copper, and coal.

In the chemical industry, physical separation methods like distillation, filtration, and chromatography are widely used for separating and purifying chemicals, solvents, and reaction products. For example, distillation is used to separate mixtures based on differences in boiling points, while chromatography aids in isolating chemical compounds from complex mixtures.

The food and beverage industry also relies heavily on physical separation techniques such as centrifugation, filtration, crystallization, and membrane processes. These methods are employed for separating solids from liquids, purifying liquids, concentrating flavors, or separating components based on solubility. They are essential in processing items such as oils, juices, dairy products, and sugars, ensuring product purity and quality.

In the pharmaceutical industry, physical separation methods like chromatography, centrifugation, and ultrafiltration are used to isolate and purify active pharmaceutical ingredients (APIs), proteins, and other biomolecules. These techniques are critical in drug development, diagnostic testing, and the production of high-quality medications.

Overall, physical separation techniques are indispensable in optimizing production processes, ensuring product quality, and improving the efficiency of operations across these diverse industrial sectors.

II.5.2 Environmental Applications

Environmental Applications of Physical Separation Methods are vital for managing and reducing environmental impacts in areas such as wastewater treatment, air purification, and recycling. These methods help minimize pollution, conserve resources, and ensure sustainable practices across various sectors.

In wastewater treatment, physical separation techniques like sedimentation, filtration, and membrane processes such as reverse osmosis are used to remove suspended solids, contaminants, and harmful bacteria from wastewater, ensuring that treated water meets environmental standards before being released back into natural water bodies. Additionally, centrifugation and flotation are commonly used to separate oil and grease from water, further improving water quality for safe disposal or reuse.

For air purification, physical separation methods like electrostatic precipitators and mechanical filters are employed to capture dust, smoke, and particulate matter from industrial emissions. These techniques help to reduce air pollution by removing harmful particles before they can be released into the atmosphere, improving air quality and protecting public health.

In recycling, physical separation is essential for sorting and processing recyclable materials. Techniques such as magnetic separation, air classification, and screening are used to separate metals, plastics, paper, and other recyclable materials from mixed waste streams. This allows for the recovery of valuable resources, reducing the volume of waste sent to landfills and promoting the circular economy. Centrifugation is also used in the recycling of materials like plastics and oils to separate contaminants.

Overall, physical separation methods are indispensable in environmental protection, enabling cleaner water, air, and land by improving waste management, promoting recycling, and controlling pollution. These techniques contribute to sustainability, helping industries comply with environmental regulations and reduce their ecological footprint.

II.5.3 Laboratory Applications

Laboratory Applications of Physical Separation Methods are crucial for sample preparation, purification, and analysis across various scientific disciplines, including biochemistry, chemistry, environmental science, and forensic analysis. These techniques help isolate, purify, and analyze components in complex mixtures, making them essential tools in both research and diagnostics.

In biochemistry and molecular biology, methods like centrifugation, chromatography, and electrophoresis are used extensively for sample preparation and purification. For example, gel electrophoresis allows for the separation of DNA, RNA, or proteins based on their size and charge, aiding in the analysis of genetic material or protein structure. Chromatography, including techniques like affinity chromatography or high-performance liquid chromatography (HPLC), is used to purify biomolecules by separating them from complex mixtures, making it essential for studying enzyme activity, protein function, and metabolic pathways.

In chemistry, distillation, filtration, and extraction are employed for purifying chemicals and isolating individual components from liquid mixtures. Distillation is particularly useful for separating liquids with different boiling points, while filtration helps remove particulates from liquids or gases, ensuring cleaner samples for further analysis. Solid-phase extraction is another technique used to isolate specific compounds from complex mixtures, crucial for environmental monitoring or pharmaceutical testing.

In environmental science, physical separation methods like filtration, sedimentation, and membrane filtration are used for sample preparation in water and soil analysis. These methods remove impurities or suspended particles, providing cleaner samples for detailed chemical or microbial analysis. In forensic science, separation techniques such as chromatography and electrophoresis are key for analyzing trace evidence, such as blood, fibers, or drug compounds, helping to identify substances in criminal investigations.

Overall, physical separation methods play a vital role in sample preparation, purification, and analysis. They enable researchers to isolate individual components from complex mixtures, providing the necessary purity and concentration for accurate and detailed scientific studies, diagnostic testing, and quality control in various laboratories.

II.6. Advantages and Limitations

Advantages and Limitations of Physical Separation Methods

Advantages of Physical Separation Methods

1. **No Chemical Reactions:** One of the main advantages of physical separation methods is that they do not involve any chemical reactions. This means that the chemical structure of the substances being separated remains unchanged, allowing for the recovery of pure, unaltered materials.
2. **Simplicity:** Many physical separation techniques are relatively simple and easy to perform, requiring basic equipment and straightforward procedures. This simplicity makes them suitable for use in a wide range of applications, from laboratory research to large-scale industrial processes.
3. **Cost-Effective:** Since physical separation techniques often do not require expensive reagents or specialized chemicals, they are typically more cost-effective than other complex methods like chemical synthesis or chromatography. This makes them ideal for large-scale applications, such as in the food, chemical, and pharmaceutical industries.
4. **Non-Destructive:** These methods generally do not alter the physical or chemical properties of the components being separated. This is particularly important when working with valuable or sensitive materials, as it allows for the recovery of pure components in their original state.
5. **Scalability:** Many physical separation techniques, such as distillation, centrifugation, and filtration, are highly scalable. They can be used in both small-scale laboratory

settings and large-scale industrial applications, making them versatile across various industries.

6. **Efficiency:** When properly applied, physical separation methods such as membrane filtration and sedimentation can achieve high separation efficiency, making them ideal for purifying or concentrating substances in research and industrial contexts.

II.7. Limitations and Challenges of Physical Separation Methods

1. **Not Suitable for All Types of Mixtures:** Physical separation methods often rely on properties such as size, density, or solubility to separate components. For mixtures where the components are chemically similar or share similar physical properties, these techniques may be ineffective or provide incomplete separation. For example, separating two substances of similar molecular size or polarity can be challenging using only physical methods.
2. **Incomplete Separation:** In some cases, physical separation methods may not fully separate all components in a mixture. Some residual impurities may remain in the separated fractions, necessitating additional purification steps. This limitation can be particularly problematic in high-precision applications like biotechnology and pharmaceuticals.
3. **Energy Consumption:** Certain physical separation techniques, such as membrane filtration, reverse osmosis, and distillation, can be energy-intensive, especially when large volumes or high pressures are involved. This can make the methods more expensive to operate on a large scale, particularly in industries like water treatment and chemical processing.
4. **Need for Specialized Equipment:** While many techniques are simple, some require specialized and often expensive equipment, such as high-performance liquid chromatography (HPLC) or gel electrophoresis. These technologies may require skilled operators, further increasing the costs and complexity of their use.
5. **Limited Resolution for Complex Mixtures:** For mixtures with a broad range of components that differ slightly in their physical properties, achieving effective separation can be difficult. For example, chromatography may struggle with separating molecules that are very similar in size or polarity, and filtration may not work well for very fine particles or colloidal suspensions.

6. Time-Consuming: Some physical separation processes, such as centrifugation or sedimentation, may take a significant amount of time to complete, especially when the differences in properties between the components are small. This can limit their effectiveness when rapid separation is required.

Chapter III: Chemical Separation and Purification Methods

III.1. Introduction

Chemical separation methods utilize chemical reactions or differences in chemical properties to separate components of a mixture. Unlike physical separation techniques, which rely on physical properties such as size, density, or solubility, chemical methods exploit factors like chemical reactivity, polarity, solubility, and molecular structure. These techniques include distillation, extraction, precipitation, chromatography, and electrochemical separation, each tailored for different types of mixtures based on their unique chemical characteristics.

The primary distinction between chemical and physical separation methods lies in the mechanism of separation. Physical methods, such as filtration, centrifugation, or distillation, do not alter the chemical composition of the components; they depend purely on physical properties. For example, in distillation, components are separated based on their different boiling points without changing their chemical structure. In contrast, chemical separation methods often involve changes in the chemical composition, such as chemical reactions or selective solubility.

Chemical separation techniques are widely used across various industries. In pharmaceuticals, they are essential for purifying drugs, isolating active ingredients, and ensuring the safety and efficacy of medications. In the petrochemical and chemical industries, they are employed for refining crude oil, producing high-purity chemicals, and separating valuable by-products. These methods are also vital in environmental science, helping to remove contaminants from water, air, and soil, contributing to cleaner, safer environments. Additionally, in biotechnology, chemical separation is crucial for isolating proteins, DNA, and other biomolecules for research and development.

The importance of chemical separation in these industries is significant, as it plays a key role in product development, quality control, waste management, and environmental protection. Effective chemical separation enables the production of high-purity substances, the purification of valuable compounds, and the efficient management of pollutants. As industries continue to advance, the need for more efficient and sustainable chemical separation methods will remain crucial to meet both industrial and environmental challenges.

III.2. Principles of Chemical Separation

Chemical separation methods are based on the fundamental concept that substances in a mixture possess distinct chemical properties, which can be exploited to isolate or purify specific components. These properties include reactivity, solubility, polarity, molecular size, and

chemical affinity. By leveraging these differences, various separation processes can be employed to isolate individual components from a mixture. Below is an overview of how these chemical properties drive separation processes:

1. **Differences in Chemical Reactivity:** The reactivity of substances plays a crucial role in separation techniques like precipitation and chemical extraction. When one component of a mixture reacts selectively with a reagent, it forms a new substance (e.g., a precipitate) that can be separated from the unreacted components. For example, in precipitation, an insoluble compound forms when two solutions are mixed, and the solid can be filtered out from the liquid.
2. **Solubility Differences:** One of the most common driving forces behind chemical separation is differences in solubility. Solubility is the ability of a substance to dissolve in a solvent, and it varies with temperature and the nature of the solvent. In liquid-liquid extraction, the solubility of one or more components in different solvents is used to separate them. For example, water-soluble compounds can be separated from oil-soluble compounds by using an appropriate solvent for each.
3. **Chemical Affinity:** Chemical affinity refers to the tendency of certain substances to interact or bond with each other. Separation methods like chromatography exploit these differences in affinity. In chromatography, components are separated based on their different interactions with a stationary phase (e.g., silica gel) and a mobile phase (e.g., solvent). Substances with higher affinity for the stationary phase move more slowly, while others that interact more with the mobile phase move faster, allowing separation based on their affinity.
4. **Boiling and Melting Points:** In distillation, the differences in the boiling points of components are utilized. By heating the mixture, the component with the lower boiling point will vaporize first, allowing it to be separated from the other components. Similarly, fractional crystallization can separate substances based on their different melting points.
5. **Chemical Reactions:** Chemical reactions are often used to drive separation by converting one or more components into a new, separable phase. In processes such as electrochemical separation, a chemical reaction causes the desired component to either precipitate or migrate toward an electrode, facilitating its separation. Redox reactions

or acid-base reactions can also be employed to change the state or solubility of specific components, enabling their isolation.

6. **Role of Solubility Rules and Chemical Affinity:** Solubility rules are crucial in many chemical separation techniques. For example, solubility rules guide the selection of appropriate solvents for extraction, ensuring that one substance dissolves in one solvent while others remain insoluble. Similarly, the chemical affinity between a solute and solvent is key in processes like membrane filtration or dialysis, where selective permeability is used to separate molecules based on their chemical interaction with the filter material.

III.3. Common Chemical Separation Techniques

Chemical separation techniques are essential for isolating specific components from complex mixtures, and several methods are commonly used depending on the nature of the substances involved. Distillation is widely used to separate liquids based on differences in boiling points. In this process, a mixture is heated, and the component with the lower boiling point evaporates first, allowing it to be collected separately. Extraction involves separating a substance from a mixture using a solvent, where differences in solubility drive the process. This method is often used for separating organic compounds from aqueous solutions. Chromatography, including methods like gas chromatography (GC) and liquid chromatography (LC), is used to separate compounds based on their interaction with a stationary phase and a mobile phase. This technique is particularly useful for purifying and identifying compounds in complex samples. Precipitation relies on the formation of an insoluble solid (precipitate) when two solutions are mixed, often driven by chemical reactions that change the solubility of one component. Electrochemical separation techniques, such as electrophoresis or electrolysis, exploit the movement of charged particles under an electric field, enabling separation based on charge and size. Other techniques like filtration and membrane separation are also commonly used, where the separation occurs based on size exclusion or selective permeability. Each of these methods utilizes the differences in chemical properties like solubility, polarity, charge, or reactivity to effectively isolate desired components for further analysis or use.

III.3.1. Extraction

Extraction is a chemical separation technique that relies on the principle of differences in solubility to separate a desired substance from a mixture. In this process, a solvent is used to selectively dissolve the target compound, based on its higher solubility in the solvent compared to other components of the mixture. The extracted substance is then separated from the

remaining mixture, either by phase separation or by evaporating the solvent. The efficiency of the extraction process depends on factors such as the solvent chosen, temperature, and the physical properties of the substances involved.

There are several types of extraction techniques, including liquid-liquid extraction, solid-liquid extraction, and supercritical fluid extraction:

- **Liquid-liquid extraction (solvent extraction):** This method involves using two immiscible liquids, typically an organic solvent and water, to separate compounds. One compound is more soluble in the organic solvent, while the other is more soluble in water. This technique is commonly used to extract organic compounds from aqueous solutions.
- **Solid-liquid extraction:** In this method, a solvent is used to extract soluble components from a solid. It is widely used in the extraction of natural products, such as essential oils, herbal compounds, or plant-based active ingredients.
- **Supercritical fluid extraction (SFE):** This technique uses a supercritical fluid, such as carbon dioxide, which has properties between a gas and a liquid, to extract compounds. SFE is especially useful in industries like food processing, where it is used to extract flavors, fragrances, and bioactive compounds from natural sources.

Extraction is extensively used in a variety of industries, including pharmaceuticals, food processing, chemical manufacturing, and environmental science. Some common applications include:

- **Separation of organic compounds:** Extraction is widely used in the pharmaceutical and chemical industries to isolate and purify organic compounds, such as alkaloids, essential oils, and active ingredients from natural sources.
- **Metal extraction:** In metallurgy, solvent extraction is often used to separate valuable metals, such as copper, gold, or uranium, from ores and waste materials.
- **Pollutant removal:** Extraction is also employed in environmental science to separate pollutants, such as heavy metals or organic contaminants, from water, soil, or air, in processes like wastewater treatment and soil remediation.

III.3.1.1. Principle

Extraction is a process used to isolate a solid or liquid component from a mixture by treating it with a solvent. It occurs in two stages: transferring the target compound to the solvent and then separating the solvent from the mixture. The principle of extraction is based on the varying solubility of different components in the mixture, allowing selective separation.

Common methods include:

- ✓ liquid-liquid extraction (Liq-Liq)
- ✓ solid-liquid extraction (Sol-Liq).

The efficiency of the process depends on factors like solvent choice, temperature, and solvent-to-mixture ratio. Repeating the process can improve the purity and yield of the extracted substance.

III.3.1.2. Liquid-Liquid Extraction

Liquid-liquid extraction is one of the oldest sample preparation techniques. It is a fundamental process for transferring a substance between two immiscible liquid phases, without any heat transfer. This technique allows for the extraction of a substance dissolved in one solvent by using another solvent, known as the extracting solvent, in which the substance is more soluble. The initial solvent and the extracting solvent must be immiscible.

III.3.1.2.1. Principle

Since water does not evaporate easily, a chemical compound dissolved in water is difficult to recover. In this case, an organic solvent in which the substance is highly soluble (much more so than in water) is used to transfer the compound from the water to the organic solvent. It is important that water and the organic solvent are immiscible.

There are two types of liquid-liquid extraction:

- **Batch Extraction:** In this method, liquid-liquid extraction is performed by vigorously shaking the solvent and the solution to be extracted in a separatory funnel.
- **Continuous Extraction:** In continuous extraction, the solution to be extracted is continuously supplied with fresh solvent, which is recycled through distillation.

III.3.1.2.2. Extraction Protocol

In the extraction protocol (figure 26), the solution to be extracted and the extraction solvent are first added to a separatory funnel. After sealing the funnel, it is shaken vigorously while being held upside down with both hands to ensure thorough mixing. This step helps reach the partition equilibrium, which is essential for optimal solute extraction. After shaking, the funnel is placed back on its stand, with the cap removed to avoid any overpressure. The phases are then allowed to separate. Once separation occurs, the two phases are carefully collected, with the aqueous phase typically being denser than the organic phase, except when chlorinated solvents are used. Proper separation of the phases ensures the extraction process is efficient and the desired component is isolated effectively.

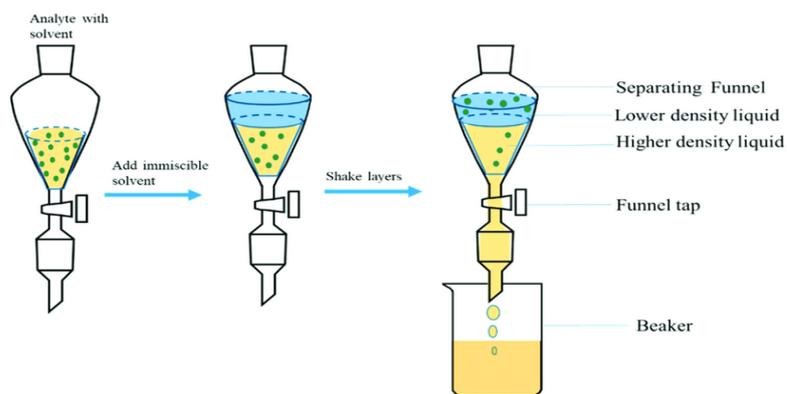


Figure 26 : Liquid-Liquid Extraction Diagram

III.3.1.3. Solid-Liquid Extraction

Solid-liquid extraction, also known as solvent extraction, is one of the oldest and most commonly used techniques. While it is often performed in everyday tasks such as brewing coffee, it also plays a crucial role in various industries, including hydrometallurgy (for selective ore dissolution or leaching), as well as in food and cosmetics production (e.g., extracting oils, sugars from beets, and natural essences). This technique involves extracting a chemical compound from a solid material and transferring it into a chosen solvent. Typically, solid-liquid extraction is carried out using a reflux heating system.

One of the challenges of solid-liquid extraction is that solids generally resist allowing liquids to pass through them. As a result, multiple extraction steps are often necessary to efficiently extract the desired compound. To address this, the Soxhlet extractor is commonly used, often in a more economical form. This specialized glassware, named after its inventor Franz von Soxhlet, is designed to perform solid-liquid extraction with great efficiency. The Soxhlet method has several advantages: it enables the sample to quickly interact with fresh solvent, helping to shift the extraction equilibrium in favor of the solvent. Additionally, the Soxhlet method does not require filtration after extraction and can be applied independently of the plant matrix.

III.3.1.3.1. Principle

The solid-liquid extraction apparatus (figure 27) is composed of several key components. It includes a flask that holds a reservoir of solvent, which is essential for the extraction process. The main extraction device, known as the extractor, ensures contact between the solvent and the solid material contained in a porous cartridge, allowing the solvent to dissolve the desired

compounds from the solid. Once the solvent has dissolved the solute, the resulting solution is evacuated back into the flask through a siphon. To support the process, a water condenser is used to condense the solvent vapors inside the porous cartridge, ensuring the solvent remains within the system for continuous extraction. This setup enables efficient extraction by facilitating repeated contact between the solvent and the solid, promoting effective solute transfer.

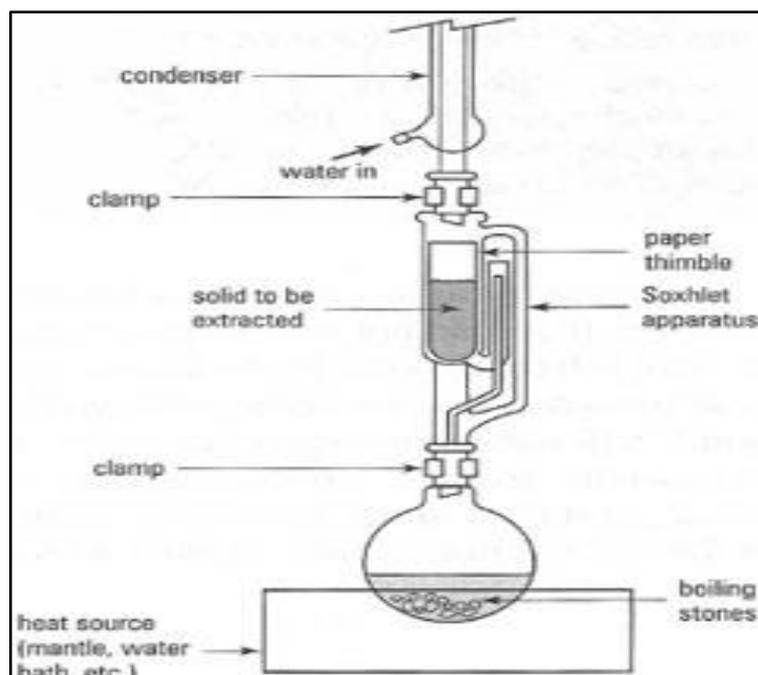


Figure 27: Solid-Liquid Extraction Diagram

III.3.1.3.2. Discontinuous extraction

Methods encompass several techniques, each suited for different types of compounds and extraction needs. Decoction involves boiling a solid in a liquid solvent, making it a fast but aggressive method best used for extracting thermally stable substances. In contrast, infusion heats the solvent without boiling it, followed by cooling, as seen in the preparation of tea. Maceration, a slower process, involves soaking the solid in a cold solvent and is often used for extracting delicate molecules, typically in a covered container to protect the mixture from light and sometimes refrigerated for added preservation. Lastly, digestion is a heated form of maceration, providing a faster option, commonly used in pharmaceuticals and perfumery. While maceration and digestion are slower, they are essential for extracting sensitive compounds without compromising their integrity.

III.3.1.3.3. Continuous Extraction

Continuous extraction is a more time-consuming process compared to discontinuous extraction, but it offers greater efficiency. One common method used in continuous extraction is percolation, which involves slowly passing a solvent through a layer of finely powdered substance. This layer is typically contained within a thick, porous paper cartridge or a filter pouch. The slow movement of the solvent ensures thorough extraction of the desired compounds, making the process highly effective for certain types of extractions, particularly when higher yields are required. Although the process takes longer, its efficiency makes it a valuable technique in various industries, including pharmaceuticals and chemical production.

III.3.1.3.4. Extraction Protocol

The extraction protocol begins by weighing the material to be extracted and placing it into the Soxhlet cartridge. Next, the solvent is introduced into the round-bottom flask and heated to initiate the extraction process. The extraction continues until the liquid surrounding the cartridge becomes clear, signaling that the solvent has extracted all the soluble components from the solid. At this point, the extraction is considered complete. This process ensures the efficient separation of desired substances from the solid material.

III.3.1.3. Hydrodistillation Extraction

Hydrodistillation involves distilling a compound by steam distillation. It is the most commonly used method for extracting essential oils. However, it has its limitations when the molecules to be extracted are delicate and cannot withstand heat.

III.3.1.3.1. Principle

The natural aromatic material (figure 28) is placed in a flask with water, which is then heated to boiling. As the heat causes the plant cells to rupture, the aromatic compounds are released and carried away by the steam. The steam passes through a condenser where it cools and condenses. The essential oil and water are then separated based on their differing densities in a separating funnel. The water, known as the distillate, retains a strong fragrance, and when derived from flowers, it is called floral water.

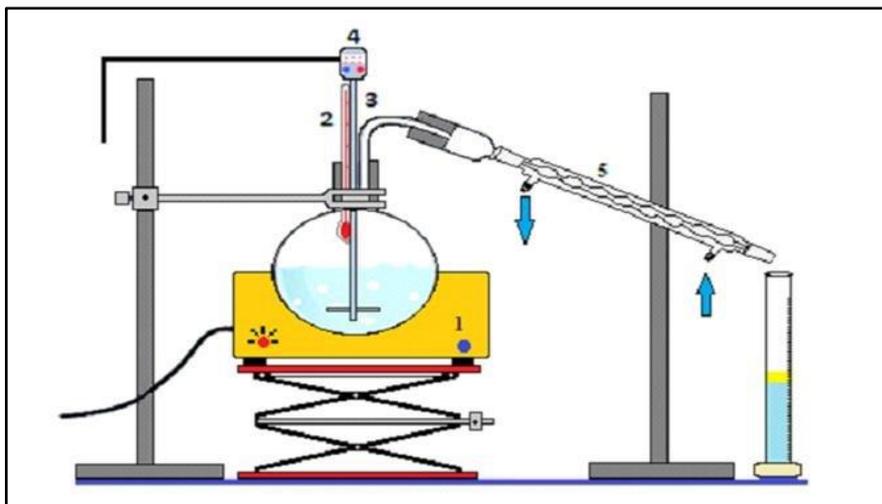


Figure 28 : Diagram of a hydrodistillation setup

III.3.2. Precipitation

Precipitation is a chemical separation technique in which an insoluble solid, known as a precipitate, forms from a solution. This occurs when the concentration of ions in a solution exceeds the solubility limit of a compound, causing it to combine and separate as a solid. The principle of precipitation is based on the formation of this solid phase from a dissolved state, driven by the solubility product (K_{sp}), which determines the point at which a substance becomes insoluble in a solution.

The principle behind precipitation involves the formation of an insoluble solid (precipitate) from a solution when the concentration of the ions exceeds the solubility limit of the product. When two solutions containing ions that can combine to form an insoluble compound are mixed, the resulting compound precipitates out of the solution. This process is driven by solubility rules and can be influenced by factors such as temperature, pH, or the presence of additional reagents.

III.3.2.1. Principle

Precipitation (figure 29) involves forming a heterogeneous phase within another phase. If the presence of certain ions is suspected in a solution, another ion can be added to form a solid substance with them. Thus, if the suspected ion is present, a solid substance will appear, which can then be filtered and collected. Precipitation is a method for separating mixtures.

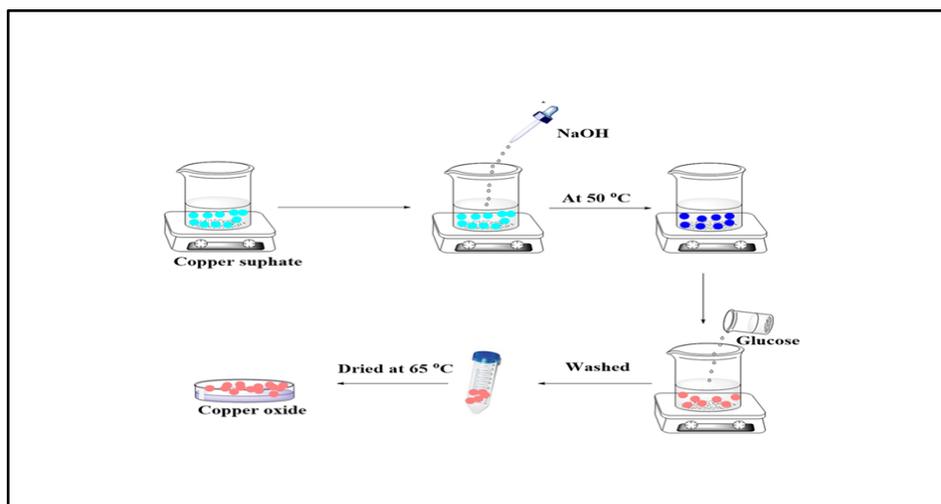


Figure 29 : Schematic representation of synthesis protocol of Cu_2O by chemical precipitation method

III.3.2.2. Methods of Precipitation

Methods of precipitation involve various techniques to selectively separate components from a solution. One common method is selective precipitation, which relies on the differences in solubility of ions within a mixture. By altering the concentration of certain ions or modifying the solution's conditions, specific ions can be precipitated out of the solution while others remain dissolved. Another technique is controlled pH adjustment, where the pH of the solution is modified to promote the precipitation of specific ionic compounds. For example, increasing or decreasing the pH can reduce the solubility of certain compounds, causing them to form solid particles that can then be separated from the solution. Both methods are widely used in various chemical processes to isolate and purify substances.

III.3.2.3. Applications of Precipitation

Precipitation has a wide range of applications in various industries. One key application is the removal of ionic impurities, where precipitation is used to eliminate unwanted ions from solutions. In water treatment, for instance, precipitation helps remove harmful ions, such as heavy metals like lead and copper, from wastewater, ensuring cleaner water. Water treatment processes also rely on precipitation to tackle contaminants like phosphate ions, which, if left unchecked, can cause eutrophication in water bodies. By adding specific reagents, these contaminants are converted into insoluble forms and can be easily removed. In the field of metal recovery, precipitation plays a critical role in extracting valuable metals from ores and

processing solutions. For example, gold and silver can be precipitated from cyanide solutions by introducing reducing agents, which convert metal ions into solid metal particles that can then be recovered. These applications demonstrate the versatility of precipitation in purifying and recovering materials across different sectors.

III.3.3. Distillation (Chemical Aspect)

Distillation is a chemical separation process that relies on differences in the boiling points of components in a mixture to separate them. The principle behind distillation is that when a liquid mixture is heated, the components with the lowest boiling points vaporize first. The vapor is then condensed back into a liquid and collected, while the remaining components with higher boiling points stay in the original container. This process can be repeated to further purify the substances.

The core principle of distillation is the separation of components based on their distinct boiling points. When a liquid mixture is heated, the component with the lower boiling point vaporizes first. This vapor is then directed into a cooler area, where it condenses back into a liquid. The difference in boiling points allows for the separation of the individual components as they condense at different temperatures. This process can be performed under normal atmospheric pressure (simple distillation) or under reduced pressure (vacuum distillation) to lower the boiling points of the substances involved.

III.3.3.1. Methods of Distillation

Distillation is a versatile separation technique with several variations, each suited to different types of mixtures. Simple distillation is used when the boiling points of the components in the mixture differ significantly. It is commonly employed to separate a volatile solvent from a non-volatile solute or to purify liquids with large differences in boiling points. In cases where the boiling points of the components are closer together, fractional distillation is the preferred method. This technique utilizes a column to facilitate multiple cycles of vaporization and condensation, enhancing the separation of components based on their boiling point differences. Vacuum distillation is employed when dealing with substances that have high boiling points or are prone to decomposition at elevated temperatures. By reducing the pressure above the mixture, this method lowers the boiling points of the components, allowing the distillation process to occur at lower temperatures, which is ideal for preserving heat-sensitive compounds

III.3.3.2. Applications of Distillation

Distillation has a wide range of applications across various industries. Purification is one of its key uses, as it effectively separates impurities based on differences in boiling points. This is essential in industries such as petrochemicals, where crude oil is refined into products like gasoline, diesel, and kerosene, as well as in pharmaceuticals for isolating and purifying chemicals. Distillation is also crucial for the separation of components in complex mixtures, such as in the production of alcoholic beverages where ethanol is separated, the extraction of essential oils, and water desalination processes that remove salts and contaminants from seawater. Additionally, chemical production relies heavily on distillation to separate reaction products from solvents or unreacted materials. It is commonly used in laboratories and manufacturing settings to prepare high-purity solvents and chemicals, ensuring precise formulations for various industrial applications.

III.3.4. Chromatography (Chemical Aspect)

Chromatography is a chemical separation technique used to separate and analyze components in a mixture based on their differential affinities for a stationary phase and a mobile phase. The principle of chromatography is based on the partitioning of substances between these two phases, with each component of the mixture moving at a different rate, allowing them to be separated.

The core principle of chromatography is the separation of components based on their differing interactions with a stationary phase (solid or liquid) and a mobile phase (liquid or gas). When the mixture is introduced into the system, the components of the mixture interact with both phases in different ways, depending on their chemical properties such as polarity, size, and solubility. Substances that have a stronger attraction to the stationary phase will move slower, while those that have a stronger affinity for the mobile phase will travel faster, leading to their separation.

III.3.4.1. Types of Chromatography

Chromatography is a versatile technique used to separate components of a mixture based on their interactions with a stationary phase and a mobile phase. Thin-Layer Chromatography (TLC) is a simple and quick method where a thin layer of stationary phase, typically silica gel, is applied to a flat surface. The mixture is spotted onto the plate, and the mobile phase, usually a solvent, moves through the stationary phase by capillary action, separating the components according to their affinity for the phases. Gas Chromatography (GC) is used for separating volatile compounds, with an inert gas as the mobile phase. The sample components are vaporized and carried through a column, where they interact with a solid or liquid stationary

phase, separating based on their affinity for the phases. High-Performance Liquid Chromatography (HPLC) employs a liquid mobile phase and a solid stationary phase, making it ideal for analyzing complex mixtures. It is commonly used in the pharmaceutical, environmental, and chemical industries to separate compounds based on their size, polarity, and solubility. Lastly, Affinity Chromatography utilizes the selective binding properties of specific molecules to isolate biomolecules like proteins, nucleic acids, and antibodies. This technique is commonly applied in biochemistry and biotechnology for purifying biomolecules based on their affinity for a ligand attached to the stationary phase.

III.3.4.2. Applications of Chromatography

Chromatography has a wide range of applications across various industries. In the pharmaceutical industry, it is essential for the purification and analysis of drugs and active pharmaceutical ingredients (APIs), enabling the separation of compounds in complex mixtures, identifying impurities, and ensuring the quality and purity of products. In environmental science, chromatographic techniques are used to detect and analyze pollutants in environmental samples, such as water, air, and soil. For example, Gas Chromatography (GC) is frequently employed to analyze volatile organic compounds (VOCs) in air samples. In biochemistry and biotechnology, chromatography is vital for protein purification, studying enzymatic activity, and isolating specific compounds. Affinity chromatography, in particular, is used to isolate biomolecules like antibodies or enzymes from complex mixtures. Additionally, chromatography is applied in forensic science for drug testing and poison analysis, as well as in the food industry, where it helps analyze flavors, additives, and contaminants in food products.

III.3.5. Ion Exchange

Ion exchange is a chemical separation process in which ions in a solution are exchanged with ions attached to a solid substrate, typically an ion-exchange resin. The principle behind ion exchange is based on the attraction between oppositely charged ions. In this process, ions of the same charge but different affinities are swapped between the solution and the solid phase, allowing for the selective removal or replacement of certain ions in a solution. The ion-exchange resin, usually made of a polymer material, contains functional groups that can either attract and hold onto positively charged ions (cations) or negatively charged ions (anions), enabling the separation of these ions from a solution.

III.3.5.1. Process

Ion-exchange processes involve the interaction between resins and solutions, typically using cation or anion exchange resins. Cation exchange resins exchange positively charged ions, while anion exchange resins swap negatively charged ions. When a solution containing ions passes through the resin bed, the ions in the solution displace the ions bound to the resin, depending on their charge and affinity for the resin material. This process is selective, as ions are exchanged based on their relative concentrations and their affinity for the resin. For example, a cation exchange resin designed to exchange sodium ions (Na^+) will swap sodium for other cations, such as calcium (Ca^{2+}) or magnesium (Mg^{2+}), from the solution. Over time, the resin becomes saturated with the unwanted ions, at which point it can be regenerated. Regeneration is done by washing the resin with a solution that has a high concentration of the ions initially bound to the resin, such as a sodium chloride solution for cation exchange, restoring the resin's capacity to continue the ion-exchange process.

III.3.5.2. Types of Ion Exchange

Ion exchange can be classified into several types, each serving different purposes in various applications. Cation exchange resins are designed to remove or replace positively charged ions (cations). A common use of cation exchange is in water softening, where calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions are replaced by sodium (Na^+) ions. On the other hand, anion exchange resins are used to exchange negatively charged ions (anions). This type of resin is commonly applied in water purification processes to remove harmful anions, such as nitrate (NO_3^-) and sulfate (SO_4^{2-}). Lastly, mixed-bed exchange combines both cation and anion exchange resins within a single column, offering a more comprehensive solution for highly purified water treatment, such as in deionization processes. This method ensures the efficient removal of both cations and anions to achieve ultra-pure water.

III.3.5.3. Applications of Ion Exchange

Ion exchange plays a crucial role in a wide range of industries and applications. In water treatment, it is widely used for both softening and deionization processes. Hard water, which contains high levels of calcium and magnesium, is treated by exchanging these ions with sodium ions, while deionization removes both cations and anions to produce ultra-pure water, essential for laboratories, pharmaceuticals, and industrial applications. Ion exchange is also vital for separating and purifying metal ions, such as in the recovery of precious metals like gold or uranium from ores. In the pharmaceutical and chemical industries, ion exchange helps purify chemical products, including antibiotics, and removes contaminants or by-products in manufacturing. Additionally, in wastewater treatment, ion exchange effectively removes toxic

metal ions and radioactive isotopes, reducing environmental impact. The food and beverage industry uses ion exchange to refine sugar and purify ingredients like amino acids and vitamins. Finally, in the nuclear industry, it is crucial for removing radioactive ions from waste streams, ensuring safe disposal and management of nuclear waste.

III.3.6. Membrane Separation Techniques

Membrane separation techniques involve the use of semi-permeable membranes that allow specific substances to pass through while blocking others, based on their size, charge, or chemical properties. These membranes selectively allow the passage of certain molecules, while rejecting others. The driving forces for separation can include pressure, concentration gradients, or electric fields, depending on the type of membrane process used. The efficiency of membrane separation depends on factors such as membrane permeability, pore size, and selectivity.

III.3.6.1. Types of Membrane Separation Techniques

Membrane filtration technologies, including reverse osmosis (RO), nanofiltration (NF), and dialysis (figure 30), are widely used for various separation and purification processes. In reverse osmosis, water is forced through a semi-permeable membrane that blocks salts, organic molecules, and other impurities, making it ideal for desalination and water purification. Nanofiltration, with membranes having smaller pores than microfiltration but larger than reverse osmosis, allows the separation of small organic molecules, divalent ions, and larger monovalent ions. This makes it especially useful in softening hard water and removing organic contaminants. Dialysis, on the other hand, is a technique where substances are separated based on their differing diffusion rates through a membrane. It is commonly used in medical applications, such as kidney dialysis, and in the purification of biopharmaceutical products. These membrane technologies offer highly effective solutions for water treatment, medical applications, and industrial purification.

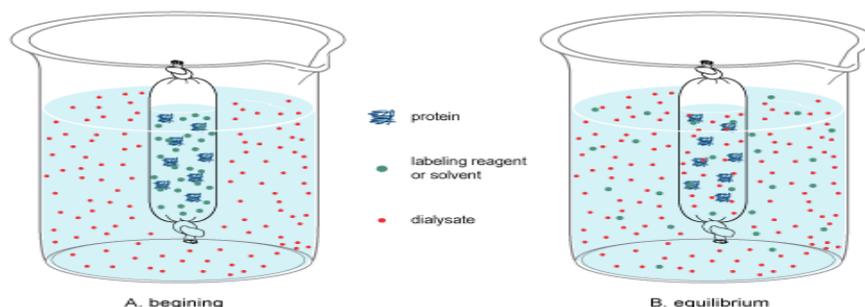


Figure 30 : Diagram of Dialysis Technique

III.3.6.2. Applications

Membrane separation techniques are widely utilized across various industries due to their efficiency and versatility. In desalination, reverse osmosis plays a crucial role in converting seawater into freshwater by effectively removing salts and impurities. In the biopharmaceutical industry, techniques like dialysis are employed to purify and separate biomolecules such as proteins and nucleic acids, ensuring the quality of pharmaceutical products. Additionally, membrane filtration is essential in wastewater treatment, where it helps remove suspended solids, bacteria, and other contaminants, allowing water to be purified for reuse or safe disposal. These techniques offer energy-efficient and cost-effective alternatives to traditional methods, making them indispensable in water treatment, pharmaceuticals, and food processing.

III.3.7. Electrochemical Separation

Electrochemical separation techniques use electric fields to manipulate the movement of charged particles (ions) within a solution. By applying an electrical current, these methods induce the migration of ions towards the oppositely charged electrodes, enabling the separation of components based on their charge and electrochemical properties. This process relies on the electrostatic interactions between ions and electric fields, leading to the selective removal, concentration, or transformation of specific components in the mixture.

III.3.7.1. Electrochemical separation techniques

Electrochemical separation techniques encompass a variety of methods that leverage electricity to separate ions and molecules in solutions. Electrodialysis (ED) uses ion-exchange membranes and an electric field to selectively separate cations and anions, making it ideal for applications such as desalination, brine treatment, and recovering valuable metals or acids from solutions. Electrolysis, on the other hand, involves passing an electric current through a solution to induce chemical reactions at the electrodes, playing a vital role in metal extraction, electroplating, and the production of chemicals like chlorine, hydrogen, and oxygen. Capacitive deionization (CDI) is a more recent technique in which ions are adsorbed onto electrodes in the presence of an electric field, offering an effective solution for desalination and water purification.

III.3.7.2. Applications

Electrochemical separation techniques are widely utilized across various industries for their efficiency and ability to handle complex mixtures. In metal refining, electrolysis is employed to extract metals like copper, aluminum, and gold from ores, as well as to purify metals in

industrial processes. In water purification, techniques like electrodialysis and capacitive deionization are integral to desalination, the removal of heavy metals, and the treatment of industrial wastewater. Electrolysis also plays a crucial role in the production of essential chemicals, including chlorine, sodium hydroxide, hydrogen, and oxygen, which are vital to chemical manufacturing and energy production. These electrochemical methods are valued for their energy efficiency, ability to separate ions effectively, and their widespread application in critical industries like metal refining, water treatment, and chemical production.

III.4. Advanced Chemical Separation Methods

Advanced chemical separation methods are highly specialized techniques used to isolate specific components from complex mixtures with high precision and efficiency. These methods often involve sophisticated technologies that exploit unique chemical properties, such as molecular affinity, solubility differences, and reactivity, to achieve selective separation. Techniques like supercritical fluid extraction, membrane filtration, and chromatography are widely used in various industries, including pharmaceuticals, environmental science, and petrochemicals. For example, supercritical fluid extraction uses supercritical fluids, typically carbon dioxide, to selectively extract compounds based on their solubility in the supercritical state, offering a greener alternative to traditional solvents. Membrane filtration, including processes like reverse osmosis and nanofiltration, utilizes semipermeable membranes to separate ions, organic molecules, and other particles from liquids. Chromatography, such as high-performance liquid chromatography (HPLC) or gas chromatography (GC), is used for separating complex mixtures by exploiting the interactions of different compounds with a stationary phase and a mobile phase. These advanced techniques enable precise separation at molecular levels, playing a crucial role in fields such as drug development, environmental monitoring, and food safety, where high purity and accuracy are required.

III.4.1. Supercritical Fluid Extraction (SFE)

Supercritical Fluid Extraction (SFE) is a separation technique that utilizes a supercritical fluid, typically carbon dioxide (CO₂), to extract desired compounds from solid or liquid samples. A supercritical fluid is a substance that is above its critical temperature and pressure, where it exhibits properties of both a liquid and a gas. In this state, the supercritical fluid has high diffusivity, low viscosity, and the ability to dissolve substances like a liquid, while also being able to permeate solids like a gas. The unique properties of supercritical fluids allow for highly efficient extraction of specific compounds, offering advantages over traditional solvents.

III.4.1.1. Method

The process involves placing the sample in a high-pressure vessel, where CO₂ (figure 31) is pressurized above its critical point (typically 31.1°C and 7.38 MPa). The supercritical CO₂ is then passed through the sample, where it dissolves the targeted compounds. Once the extraction is complete, the pressure is reduced, causing the CO₂ to return to its gaseous state, leaving behind the extracted compounds in a concentrated form. The solvent can be easily separated and reused, making SFE an environmentally friendly and cost-effective technique.

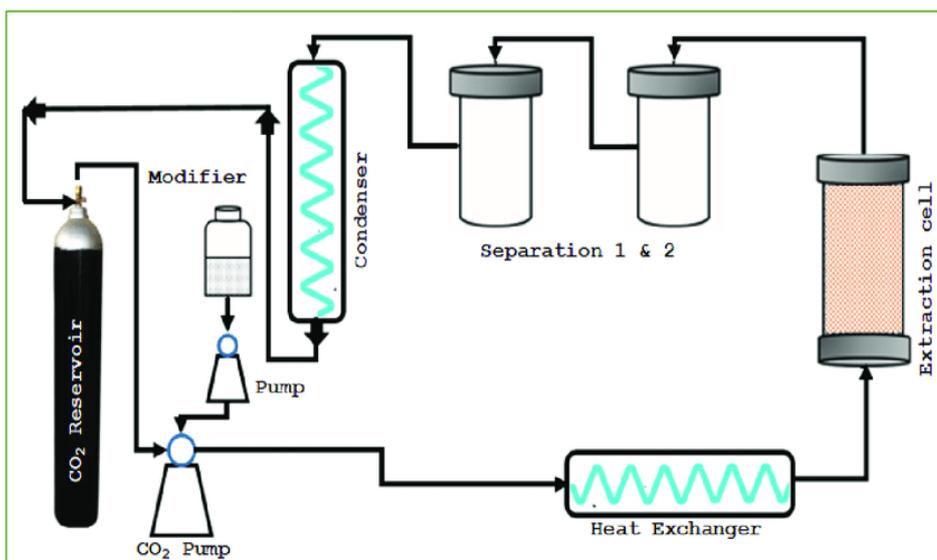


Figure 31: Illustrative diagram of supercritical fluid extraction

III.4.1.2. Applications

Supercritical Fluid Extraction (SFE) has a wide range of applications across various industries. In the pharmaceutical sector, SFE is used to extract bioactive compounds, essential oils, and natural products from plants and herbs, playing a crucial role in drug development and nutraceuticals. In the food and beverage industry, SFE extracts flavors, fragrances, and essential oils from natural sources, and is also used to decaffeinate coffee and tea without harmful solvents. In environmental science, SFE is employed to extract pollutants and contaminants from environmental samples, such as soil and water, offering a cleaner and more efficient alternative to traditional methods. In petrochemicals, SFE is used for extracting valuable components from crude oil and purifying specialty chemicals. The cosmetic industry also benefits from SFE, using it to extract natural ingredients and fragrances for skincare and beauty products. The method is considered "green" due to the use of CO₂, a non-toxic and environmentally friendly solvent, making it a more sustainable alternative to traditional solvent-based extraction techniques.

III.4.2. Affinity Separation

Affinity separation is a technique that relies on the specific interaction between a target molecule and a particular binding agent, such as an antibody, ligand, or receptor. This method exploits the high specificity of molecular interactions, where the target compound has a strong affinity for the binding agent, allowing for selective isolation from a complex mixture. The principle of affinity separation is based on the fact that the target substance binds selectively to the affinity material, while other components in the mixture are either washed away or separated through various means. Once the target molecule is bound, it can be eluted (or released) from the binding agent by changing the conditions, such as altering the pH, temperature, or ionic strength, making it highly selective and efficient.

III.4.2.1. Methods

Affinity chromatography encompasses several methods of affinity separation, each utilizing specific interactions to isolate target molecules. One of the most common techniques is affinity chromatography itself, where a column is packed with a matrix covalently bonded to a ligand that specifically binds to the target molecule. As the mixture flows through the column, only molecules that bind to the ligand will adhere to the matrix, while other components pass through. The bound molecules can then be eluted by changing the conditions to disrupt the ligand-target interaction. Another related technique is immunoaffinity separation, which employs antibodies or antigens as binding agents, such as in immunoaffinity chromatography where antibodies bind specifically to target antigens. This approach is often used for the purification of proteins, peptides, or nucleic acids. Finally, receptor-ligand interaction-based affinity separation uses receptors that specifically bind to their corresponding ligands, a method frequently applied in the purification of biologically significant molecules like hormones, growth factors, and enzymes. These affinity-based techniques offer highly selective methods for isolating and purifying target molecules in various scientific and industrial applications.

III.4.2.2. Applications

Affinity separation is widely utilized across various industries and fields due to its high specificity and efficiency. In biotechnology and biopharmaceuticals, it plays a vital role in the purification and isolation of proteins, antibodies, enzymes, and other biomolecules, and is crucial in monoclonal antibody production, where antibodies are selected to bind to specific antigens for targeted disease therapies. In water treatment and environmental sciences, affinity separation techniques are employed to remove specific pollutants or toxins from water and other environmental samples, effectively capturing harmful substances through selective binding. The food and beverage industry also benefits from affinity separation, using it to isolate

bioactive compounds such as vitamins, antioxidants, and flavors, thereby enhancing product quality and nutritional value. Additionally, in medical diagnostics, affinity separation is used to isolate biomarkers like disease-related proteins or DNA/RNA sequences, aiding in early detection and monitoring of medical conditions. The method is highly valued for its ability to isolate specific components even from complex mixtures with minimal interference from other substances, though its success relies on the availability of suitable affinity agents and optimized conditions for elution and recovery.

III.4.3. Supercritical Fluid Chromatography (SFC)

Supercritical Fluid Chromatography (SFC) is a chromatographic technique that uses a supercritical fluid as the mobile phase to separate compounds based on their chemical properties. A supercritical fluid is a substance that is at a temperature and pressure above its critical point, where it exhibits properties of both a liquid and a gas. In SFC, the most commonly used supercritical fluid is carbon dioxide (CO_2), although other gases like nitrogen or water can also be used depending on the application. The supercritical fluid has high diffusivity, low viscosity, and the ability to dissolve non-volatile compounds, making it an ideal medium for chromatographic separations.

In SFC, the sample mixture is injected into a chromatographic column, where the supercritical fluid flows through the column, interacting with the stationary phase (usually a solid or liquid adsorbent). The components of the sample mixture interact differently with the stationary phase, and as a result, they are separated based on their different affinities for the stationary phase. The separated compounds are then detected by various detectors, such as ultraviolet (UV) or mass spectrometry (MS).

III.4.3.1. Methods

Supercritical Fluid Chromatography (SFC) is a powerful technique that utilizes a supercritical fluid as the mobile phase to separate components in a mixture. The basic (figure32) setup consists of a supercritical fluid pump, a column packed with a stationary phase such as silica or polymeric material, and a detector to analyze the eluted components. The pressure and temperature are carefully regulated to ensure that the mobile phase remains in the supercritical state, which allows for unique separation properties. SFC can be further modified to suit specific applications by adjusting the composition of the mobile phase, as well as the pressure and temperature conditions. The addition of co-solvents, like methanol or ethanol, to the supercritical CO_2 is a common modification that enhances the selectivity and efficiency of the separation process, enabling better resolution and faster analysis of complex samples.

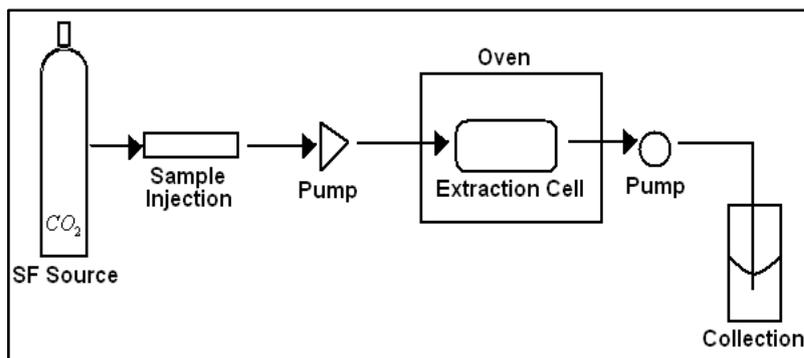


Figure 32: Scheme of a supercritical fluid chromatography instrument.

III.4.3.2. Applications

Supercritical Fluid Chromatography (SFC) has diverse and valuable applications across several industries. In the pharmaceutical industry, SFC is particularly useful for the separation and purification of pharmaceutical compounds, especially in the analysis of chiral compounds. It plays a crucial role in enantiomeric separations, enabling the isolation of a single enantiomer from a racemic mixture, which is essential for the development of drugs with specific biological activity. In the chemical industry, SFC is employed to analyze complex organic compounds, including the separation of high molecular weight compounds and the purification of polymers or small molecules. It offers a more environmentally friendly alternative to traditional solvent-based methods, reducing the ecological footprint. SFC is also widely used in environmental analysis, where it aids in the extraction and analysis of contaminants like pesticides and volatile organic compounds (VOCs) from water or air samples, providing a more efficient and eco-conscious solution compared to conventional extraction techniques. In the food and beverage industry, SFC helps analyze and separate flavor compounds, essential oils, and fatty acids, enhancing food quality and safety. Additionally, in the cosmetics and fragrance industries, SFC is used for purifying essential oils and separating fragrance components, ensuring the production of high-quality products.

III.4.4. Reactive Distillation

Reactive distillation is an advanced separation process that combines chemical reactions and distillation in a single unit operation. In this process, chemical reactions and separation of components via distillation occur simultaneously within the same column. The principle of reactive distillation is based on the fact that the distillation column not only separates the components of a mixture based on their volatility but also drives a chemical reaction between certain components of the mixture as they pass through the column.

The reactive distillation column typically consists of a series of trays or packing material where both the separation of volatile components and the desired reaction take place. The temperature and pressure within the column are carefully controlled to favor both the reaction and the distillation. Reactive distillation is often used when the reaction involved is reversible and can be driven to completion by the separation of one of the products through distillation.

III.4.4.1. Methods

Reactive distillation (figure 33) is a process that combines chemical reactions with distillation to achieve both reaction and separation in a single operation. One common method is the single-column configuration, where a single distillation column is used to perform both the reaction and separation processes. In this setup, reactants are introduced into the column, and the desired products are separated based on their volatility, either as distillates or residues. Catalysts are typically employed to enhance the rate of reaction in reactive distillation. These catalysts can be homogeneous, dissolved in the liquid phase, or heterogeneous, immobilized on the packing or trays within the column. The efficiency of the process is heavily influenced by precise control of temperature and pressure, as these factors directly impact both the distillation and reaction rates, ensuring optimal performance and product yield.

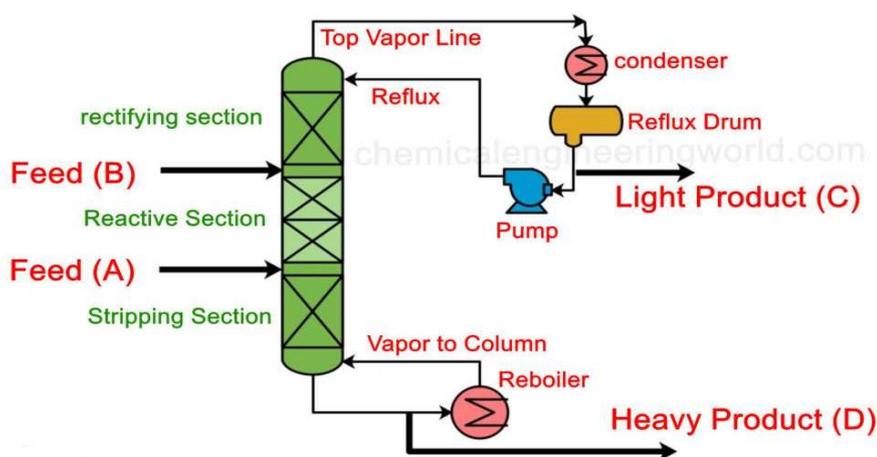


Figure 33: Reactive distillation

III.4.4.2. Applications

Reactive distillation is widely used in various industrial applications due to its efficiency in combining chemical reactions and separation processes. One prominent example is the production of Methyl Tert-Butyl Ether (MTBE), an oxygenate compound commonly used in

gasoline. In this process, isobutene and methanol react in the column, and MTBE is separated through distillation. Reactive distillation is also frequently applied in esterification reactions, such as the production of biodiesel, where alcohols react with acids to form esters, and the separation of water from the reaction mixture drives the equilibrium toward ester formation. In acid-base reactions, reactive distillation is used to produce anhydrous acids or synthesize solvents, with distillation helping to remove water or other byproducts that could impede the reaction. The method is also employed in the synthesis of acetals and ketals, which are valuable solvents, fuel additives, or intermediate chemicals. Furthermore, in biofuel production, reactive distillation plays a key role in synthesizing biodiesel by reacting oils or fats with methanol or ethanol, while simultaneously separating the glycerol byproduct.

III.5. Applications of Chemical Separation Methods

Chemical separation methods are widely used across various industries to purify, isolate, and concentrate valuable components or to remove undesirable impurities. In the pharmaceutical industry, these methods are essential for drug purification, the synthesis of active pharmaceutical ingredients (APIs), and the separation of compounds during drug formulation. Petrochemical industries rely on chemical separation techniques, such as distillation and solvent extraction, for refining crude oil, separating hydrocarbons, and producing fuels, lubricants, and chemical feedstocks. In environmental science, chemical separation is critical for wastewater treatment, air purification, and the removal of pollutants like heavy metals, organic compounds, and toxins from water and soil. In the food and beverage industry, separation methods are used to isolate flavor compounds, preservatives, and nutrients, as well as to clarify liquids, such as juices and oils. Additionally, chemical separation methods are employed in mining for extracting metals from ores, in biotechnology for protein purification and enzyme isolation, and in chemical production for purifying raw materials and controlling product composition. These techniques enable the production of high-purity substances, which is essential for product quality, regulatory compliance, and efficient resource utilization across these industries.

III.5.1. Industrial Applications

Chemical separation methods play a pivotal role in several industries, facilitating the extraction, purification, and processing of valuable components. In chemical manufacturing, techniques like distillation, filtration, and solvent extraction are used to purify raw materials, separate chemical mixtures, and refine final products. For example, in petrochemical refining,

distillation towers separate crude oil into fractions like gasoline, diesel, and jet fuel, while other methods like solvent extraction help isolate specific chemicals for use in manufacturing.

In the pharmaceutical industry, chemical separation techniques such as chromatography, precipitation, and membrane filtration are essential for the production of high-purity active pharmaceutical ingredients (APIs). These methods are crucial for removing impurities and ensuring the safety and efficacy of drugs, including the production of biopharmaceuticals and vaccines. In food processing, chemical separation methods are employed to clarify liquids, extract flavor compounds, and separate essential oils from plants. Techniques such as supercritical fluid extraction (SFE) are used to obtain bioactive compounds, while filtration and distillation help purify and concentrate products.

Moreover, chemical separation is vital in bioactive compound extraction, such as the isolation of antioxidants, vitamins, and other health-promoting compounds from natural sources. These bioactive compounds are then used in the production of dietary supplements, nutraceuticals, and functional foods.

In mining, chemical separation techniques like flotation and leaching are crucial for extracting valuable metals from ores, while in environmental applications, these methods are used for wastewater treatment, air purification, and the removal of contaminants from soil and water. Overall, chemical separation methods are integral across industries, driving efficiency, quality control, and the production of valuable substances while ensuring sustainability and environmental protection.

III.5.2. Environmental Applications

Chemical separation methods are essential in tackling environmental issues by removing pollutants and contaminants from air, water, and soil, thus supporting environmental protection and sustainability. In air purification, chemical separation plays a key role in reducing harmful emissions such as sulfur dioxide, nitrogen oxides, and volatile organic compounds (VOCs). Techniques like adsorption, where contaminants adhere to solid surfaces, and scrubbing, where chemicals neutralize harmful gases, are widely used to treat industrial exhaust gases and improve air quality.

In water purification, chemical separation methods such as ion exchange, filtration, and chemical coagulation are crucial for removing heavy metals, organic pollutants, and pathogens from water. For example, in municipal water treatment plants, chemical flocculation is employed to aggregate particles and contaminants into larger clumps, making them easier to

remove through sedimentation or filtration. These methods are vital in ensuring safe drinking water and in treating wastewater before it is released back into the environment.

Waste treatment also relies heavily on chemical separation methods. In both industrial and municipal waste management, techniques such as precipitation and solvent extraction are used to remove toxic chemicals, heavy metals, and other contaminants from waste streams. For instance, chemical precipitation is commonly used to treat wastewater containing heavy metals, where the metals are converted into insoluble compounds that can be easily removed from the water.

Additionally, pollution control involves the application of chemical separation techniques to reduce environmental contaminants. Methods like chemical oxidation and reduction are used to break down pollutants in air, water, and soil, while selective separation processes help isolate harmful substances for safe disposal or recycling. In the case of soil remediation, chemical treatments such as solvent extraction and bioremediation techniques remove or neutralize hazardous substances like pesticides and hydrocarbons, allowing the land to be safely restored for use.

Overall, chemical separation methods are fundamental to air and water purification, waste treatment, and pollution control, enabling the efficient removal of pollutants and supporting efforts to protect natural resources and ecosystems. These methods help ensure cleaner air, safer water, and healthier environments, contributing to global sustainability and public health.

III.5.3 Laboratory and Analytical Applications

Chemical separation methods are crucial in laboratory and analytical applications, enabling the efficient isolation, identification, and purification of specific components from complex mixtures. These techniques are fundamental in fields such as biochemistry, pharmaceuticals, environmental science, and chemical research. In chemical analysis, methods like chromatography, distillation, and precipitation are vital for separating and identifying compounds. Techniques such as gas chromatography (GC) and high-performance liquid chromatography (HPLC) are commonly used to analyze volatile and non-volatile compounds in samples like blood, urine, and food products, separating them based on their chemical properties and helping to identify contaminants, pollutants, or active ingredients with high precision. Sample purification is another key area where chemical separation plays an important role. Methods like extraction, recrystallization, and filtration are used to isolate desired substances from impurities, ensuring the purity of compounds in pharmaceutical research, clinical testing, and drug development. For instance, liquid-liquid extraction is often employed to isolate bioactive compounds, while recrystallization purifies solids based on solubility

differences. In scientific research, these methods are indispensable for isolating molecules, studying their properties, and conducting experiments. Techniques such as affinity chromatography and ion-exchange chromatography are used in protein purification, allowing researchers to isolate specific proteins or enzymes for further study. Similarly, in environmental research, liquid-liquid extraction helps isolate pollutants from water, soil, or air samples for analysis. These separation methods are also critical in quality control, ensuring that products in the pharmaceutical, food, and chemical industries meet regulatory standards. Overall, chemical separation methods are essential for advancing scientific discovery, improving product quality, and ensuring safety across various industries.

III.6. Advantages and Limitations

Chemical separation methods play a vital role in industries and research, offering distinct advantages while also facing certain limitations.

III.6.1. Advantages

The advantages of chemical separation methods include:

1. **High Purity:** Techniques such as chromatography and distillation allow for the isolation of compounds with a high level of purity, which is essential in industries like pharmaceuticals where strict purity standards are required for drug formulation and quality control.
2. **Selective Separation:** These methods enable the targeted separation of specific components based on their unique chemical properties, such as solubility, boiling point, or charge. This selectivity is especially valuable in applications like protein purification or the extraction of specific compounds from complex mixtures, such as natural sources.
3. **Handling Complex Mixtures:** Chemical separation methods are particularly effective for separating complex mixtures that cannot be easily divided using physical methods. They can simultaneously isolate multiple components, even when these components share similar physical properties, making them invaluable in fields like biotechnology, environmental science, and food processing.

III.6.2. Limitations

The limitations of chemical separation methods include:

1. **Cost:** Many techniques, such as high-performance liquid chromatography (HPLC) and supercritical fluid extraction (SFE), involve expensive equipment, specialized reagents, and require skilled personnel. This makes them costly, which can limit their use, particularly in small-scale operations or research environments.
2. **Environmental Impact:** Some chemical separation methods, especially those using hazardous solvents or consuming large amounts of energy (e.g., distillation), can have considerable environmental consequences. The disposal of chemical waste, reliance on non-renewable resources, and the carbon footprint of energy-intensive processes need careful management to reduce their negative effects on the environment.
3. **Complexity:** Certain chemical separation techniques, such as reactive distillation or affinity chromatography, can be technically demanding. These methods often require a deep understanding of the system being separated and may involve multiple steps to achieve the desired purity or separation. This complexity can reduce efficiency and increase the risk of errors, particularly in large-scale applications.

Chapter IV: Purification Methods in Biological Systems

IV.1. Introduction

Purification in chemistry and biology refers to the process of isolating a specific substance from a mixture and removing impurities, which can be physical, chemical, or biological in nature. In chemistry, this often involves techniques designed to separate and refine compounds, ensuring their purity for further analysis, production, or consumption. In biology, purification is crucial for isolating specific proteins, enzymes, or nucleic acids from complex biological samples.

Purification plays a critical role in many industries, including pharmaceuticals, chemical manufacturing, food production, and environmental science. In pharmaceuticals, the purification of active ingredients ensures the safety and effectiveness of medications, while in chemical manufacturing, it guarantees that products meet quality standards. In environmental science, purification methods are used to remove pollutants from water, air, and soil, contributing to environmental protection and public health.

Impurities that may be present in a substance can be broadly categorized into physical impurities (e.g., dust, dirt, or insoluble particles), chemical impurities (e.g., residual solvents, unwanted chemicals, or byproducts), and biological impurities (e.g., bacteria, viruses, or other microorganisms). The choice of purification method depends on the nature of these impurities and the desired outcome, with techniques such as filtration, distillation, chromatography, and crystallization being commonly employed to achieve high levels of purity.

In this chapter, we will focus on purification methods for biological systems.

IV.2. Purification in Biological Systems

Purification in biological systems refers to the process of isolating specific biomolecules, such as proteins, enzymes, nucleic acids, or other cellular components, from complex biological mixtures. This process is vital for studying the properties and functions of these biomolecules and for producing them for therapeutic or industrial applications. Biological purification plays a critical role in fields such as biotechnology, medicine, and research, where high purity and functionality are essential for accurate analysis, drug development, and production.

The purification process typically involves several stages, including homogenization, centrifugation, filtration, chromatography, and electrophoresis. These techniques are tailored to the specific properties of the target molecule. For example, proteins are often purified using affinity chromatography, which relies on the specific binding interactions between the target

protein and a ligand, or size-exclusion chromatography, which separates molecules based on their size. Nucleic acids are typically purified using methods such as silica-based column chromatography or precipitation techniques.

Biological purification is crucial for removing contaminants such as other proteins, lipids, nucleic acids, or microorganisms, which could interfere with downstream applications or analyses. It is also essential for ensuring the safety and efficacy of biopharmaceuticals, as impurities in biological products can lead to adverse effects. Moreover, in research, purified biomolecules are necessary for structural studies, enzymatic assays, and understanding molecular mechanisms in living organisms.

IV.2.1. Purification of Biomolecules

The purification of biomolecules, including proteins, nucleic acids (DNA and RNA), and enzymes, is a critical process in both research and industrial applications. It involves isolating a specific biomolecule from a complex mixture, such as cell lysates, tissues, or biological fluids, to study its structure, function, and potential applications. This process is essential in fields like biotechnology, medicine, and pharmaceutical development, where high purity is required for further analysis or therapeutic use. The purification of proteins, in particular, is crucial in the production of therapeutic drugs, such as monoclonal antibodies and recombinant proteins. Similarly, purified nucleic acids are essential for applications such as cloning, sequencing, and gene expression studies.

The isolation of enzymes also plays a vital role, allowing their use in various applications, including diagnostics, drug production, and even environmental bioremediation processes.

To achieve the desired level of purity, various purification techniques are employed, often in combination. Affinity chromatography is a highly specific technique that exploits the unique binding interactions between a biomolecule and a ligand, enabling the targeted purification of a protein or enzyme. Ion-exchange chromatography separates biomolecules based on their charge, using a charged stationary phase to attract or repel charged molecules. Size-exclusion chromatography is another common method that separates molecules based on their size, allowing larger molecules to elute first. Gel electrophoresis is primarily used for nucleic acids and proteins, where an electric field drives the migration of molecules through a gel matrix, with separation based on size and charge.

Purification in biological systems involves isolating specific biomolecules, such as proteins, enzymes, nucleic acids, or other cellular components, from complex biological mixtures. This process is crucial for studying the properties and functions of these biomolecules, as well as for producing them for therapeutic or industrial purposes. Biological purification plays a vital role in fields like biotechnology, medicine, and research, where high purity and functionality of molecules are necessary for accurate analysis, drug development, and production.

The purification process typically involves multiple steps, including homogenization, centrifugation, filtration, chromatography, and electrophoresis, each tailored to the specific properties of the target molecule. For instance, proteins are often purified using techniques such as affinity chromatography, which exploits the specific binding interactions between the target protein and a ligand, or size-exclusion chromatography, which separates molecules based on their size. In contrast, nucleic acids are commonly purified using methods like silica-based column chromatography or precipitation techniques.

In this context, purification is essential for removing contaminants, such as other proteins, lipids, nucleic acids, or microorganisms, which could interfere with downstream applications or analyses. It is also crucial for ensuring the safety and efficacy of biopharmaceuticals, as impurities in biological products can cause adverse effects. Moreover, in research, purified biomolecules are necessary for structural studies, enzymatic assays, and understanding molecular mechanisms in living organisms (figure 34).

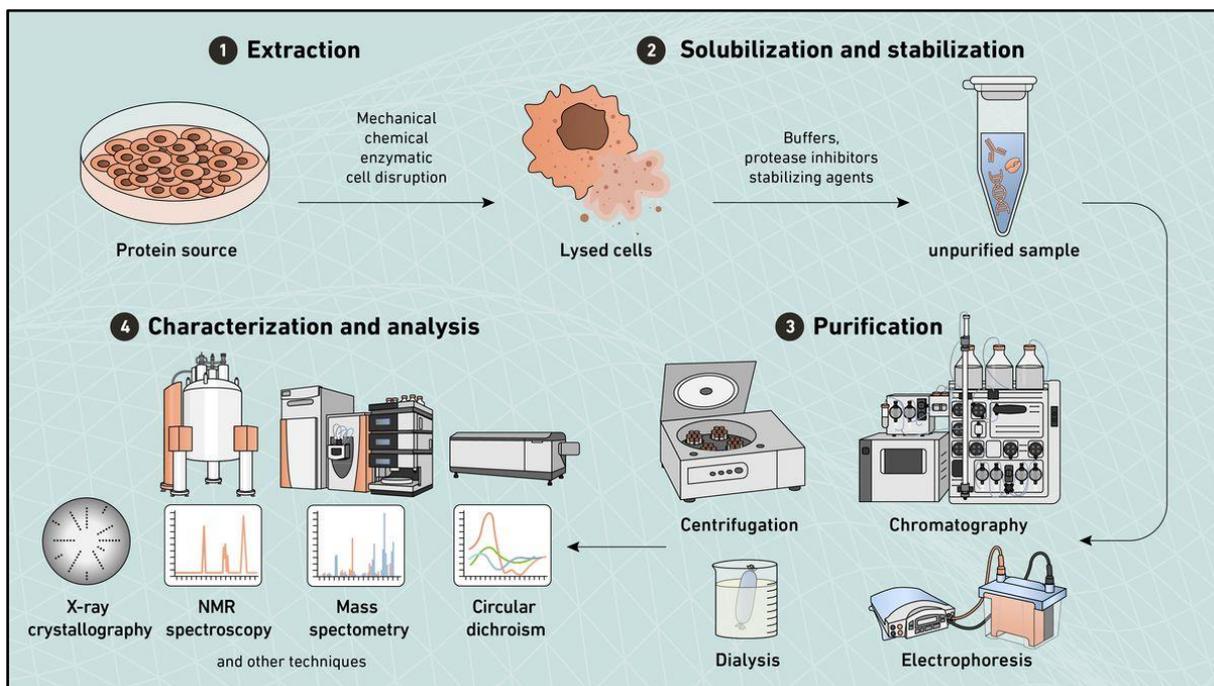


Figure 34 : Workflow of a typical protein purification procedure

Biomolecules are purified using chromatography techniques that separate them based on differences in their specific properties as shown in figure 35 and table 3. For example, ion-exchange chromatography (IEX) separates biomolecules according to their net surface charge. This approach is used in various applications, such as biological research to develop reagents like enzymes and antibodies that serve as molecular biology tools, or in diagnostics, where purified proteins are used to develop tests for disease detection. Additionally, purified proteins play a key role in areas like environmental monitoring, food and cosmetic product safety, forensic science, and biopharmaceutical development, particularly in the production of therapeutic proteins and vaccines.

Table 3: Specific property and technique

Property	Technique
Charge	Ion exchange chromatography (IEX)
Size	Size exclusion chromatography (SEC), also called gel filtration (GF)
Hydrophobicity	Hydrophobic interaction chromatography (HIC) Reversed phase chromatography (RPC)
Biorecognition (ligand specificity)	Affinity chromatography (AC)

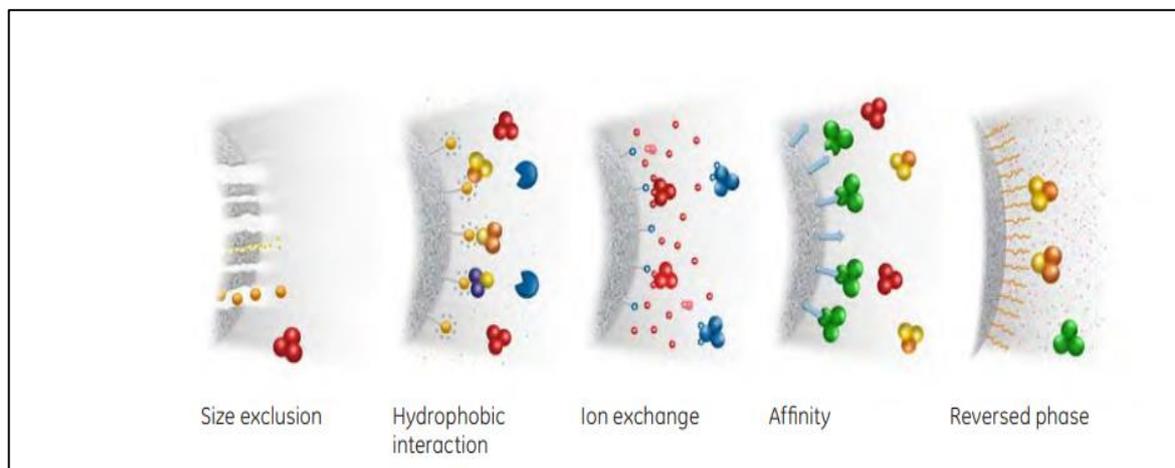


Figure 35 : Separation principles in chromatographic purification.

IV.2.1.1. Affinity chromatography

Also known as Bio-chromatography or Bio-recognition chromatography, this technique is a form of adsorption chromatography in which the target molecule selectively adsorbs to the stationary phase. Unlike other chromatography methods that use a general-purpose support, affinity chromatography requires a support specifically tailored to the molecule being purified. The stationary phase consists of a chemically inert, porous macromolecular material (such as carboxymethyl cellulose derivatives, polyacrylamide gel, silica, or agarose), to which an organic molecule (known as the effector) with selective affinity for certain components of the sample is covalently bound.

Only the target molecule will bind to this stationary phase, and it can then be eluted in two ways:

- Non-specifically, by altering the pH or ionic strength, which modifies the interactions between the molecule and the effector.
- Specifically, by introducing another molecule that competes with the effector for binding to the target molecule.

The ligand's arm, which distances it from the support, enhances the interaction between the ligand and the target molecule. To minimize non-specific interactions, high ionic strength is often used to weaken the binding of non-target molecules. In this method, the stationary phase is a chemically inert macromolecular support with a covalently attached effector, which exhibits a biological affinity for a specific solute in the sample.

Three primary types of affinity are used in this technique:

- Enzyme-substrate affinity
- Ligand-receptor affinity
- Antigen-antibody affinity

In many cases, the fixed molecule serves as the substrate, ligand, or antibody, enabling the purification of enzymes, receptors, or antigens, respectively. The specific molecule attached (figure 36) to the stationary phase allows for the targeted isolation of the corresponding molecule. The table below summarizes the various ligands separated using affinity chromatography.

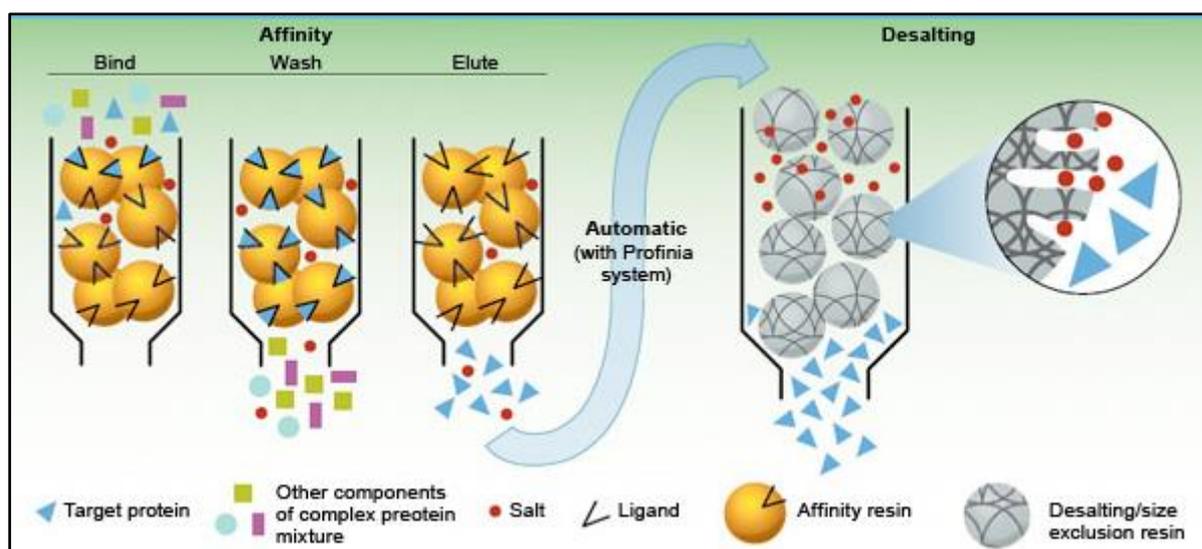


Figure 36: Affinity-tagged purification.

IV.2.1.2. Ion-exchange chromatography

Ion exchange chromatography (IEX) is a fast, economical, and versatile technique for effectively separating ions, amino acids, peptides, nucleotides, nucleic acids, and other biomolecules. It is widely used in the pre-fractionation or purification of target proteins from crude biological samples. Introduced in the 1960s, IEX has become a cornerstone technique for purifying proteins, peptides, nucleic acids, and other charged biomolecules. Its high resolution, excellent group separations, and substantial loading capacity make it particularly useful for separating molecules with minor differences in charge properties, such as two proteins differing by just one charged amino acid.

IEX is well-suited for various stages of protein purification, including capture, intermediate purification, and polishing. It is effective for both microscale purification and large-scale processes, allowing for the purification of kilograms of product. The technique operates on the principle of ionization and the charge of molecules in relation to pH, which is essential for understanding how proteins or other biomolecules will interact with the ion exchange resin.

Today, IEX continues to be widely used due to its versatility and efficiency. This handbook provides both theoretical and practical insights into IEX, covering the principles of the technique, available chromatography resins, and how to select the appropriate resin for specific applications. It also includes application examples, detailed instructions for commonly performed procedures, and practical tips drawn from over 50 years of chromatography experience. GE Healthcare's Life Sciences division offers a wide range of prepacked columns and ready-to-use chromatography media, making the purification process simple and efficient for laboratories of all sizes.

IV.2.1.2.1. Principle of Ion Exchange Chromatography

Ion exchange chromatography separates molecules, such as proteins, based on differences in their accessible surface charges (figure 37). In this technique, the analyte molecules are retained on the column through Coulombic (ionic) interactions with the stationary phase, which contains ionic functional groups of opposite charge. The elution of the analyte is achieved by gradually increasing the salt concentration, typically using sodium chloride (NaCl), which dissociates into Na⁺ (cation) and Cl⁻ (anion) in aqueous solution. As the salt concentration increases, so does the concentration of Na⁺ and Cl⁻. The core principle of ion exchange chromatography is the reversible exchange of analyte ions, which are bound to a solid support, with similar ions generated from the salt in the liquid phase.

Many biological molecules, including proteins, amino acids, nucleotides, and other ions, contain ionizable groups that carry a net charge (positive or negative) depending on their pKa and the pH of the solution. This charge can be exploited to separate mixtures of such molecules, as further explained below. Ion exchange chromatography is typically carried out in columns packed with ion exchangers. Based on the type of exchanger used, this method can be divided into two main categories: cation exchange chromatography and anion exchange chromatography.

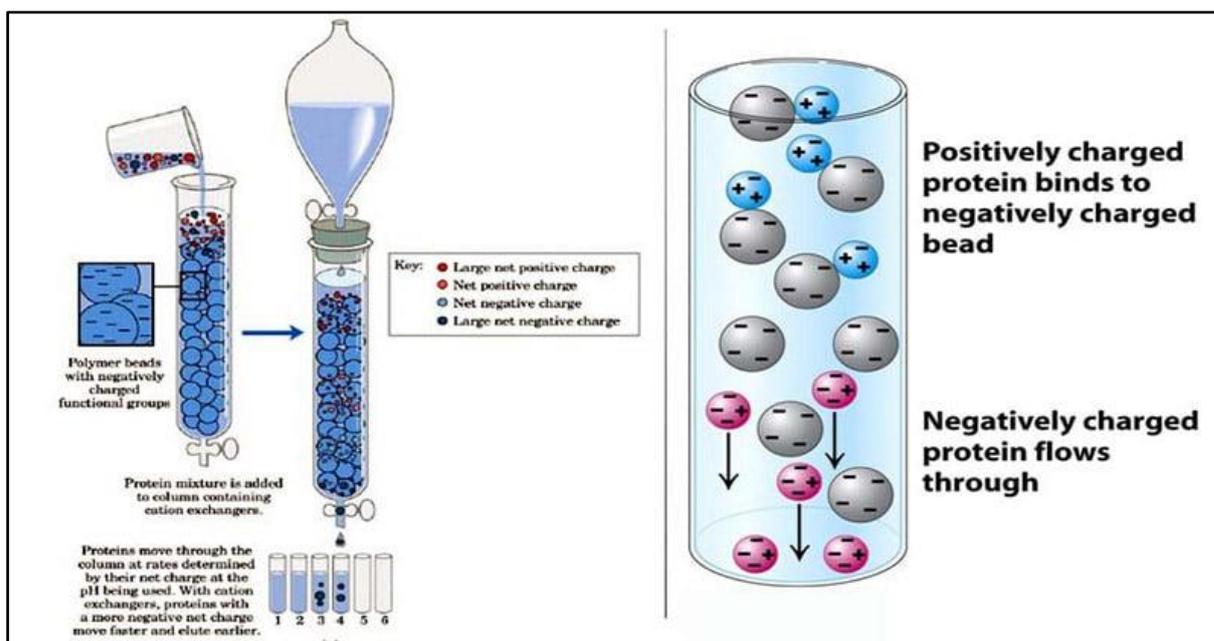


Figure 37: Principle of ion exchange.

IV.2.1.2.2. Selection of Ion Exchanger Based on pH

The stability of biological molecules, especially proteins, is often restricted to a narrow pH range, so the choice of ion exchanger depends on this stability. If a protein is most stable below its isoelectric point (pI), it will have a net positive charge. In this case, a cation exchanger should be used, with the experimental pH set between the lowest pH where the protein is stable and its pI. Conversely, if the protein is most stable above its pI, it will carry a net negative charge, and an anion exchanger should be used, with the pH set between the highest pH where the protein is stable and its pI. For proteins that are stable across a broad pH range, either type of ion exchanger can be used, with the experimental pH chosen based on the protein's stability at both ends of the pH range.

Weak electrolytes, which require very high or low pH for ionization, should be separated using strong exchangers, as they operate across a broader pH range. On the other hand, strong electrolytes are best separated using weak exchangers.

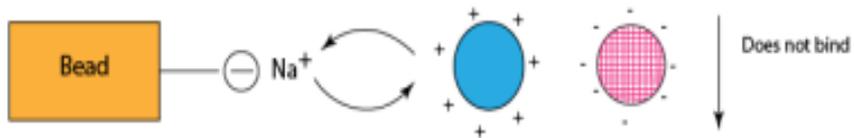
IV.2.1.2.3. Cation Exchange Chromatography

Cation exchange chromatography uses solid supports with functional groups that interact with positively charged molecules. These supports are often prepared using various types of beads, which differ in properties such as flow rate, stability, and binding capacity, with the latter being related to their porosity. Common cation exchangers include matrices based on dextran (Sephadex), agarose (Sephacrose), and cross-linked cellulose (Sephacel), all of which have high porosity. This high porosity enhances flow properties and increases the binding capacity for macromolecules. The chromatography process is typically carried out using buffers with pH values between 4 and 7. The elution is performed by running a gradient, starting from a solution containing only the buffer and gradually increasing to one containing 1M NaCl.

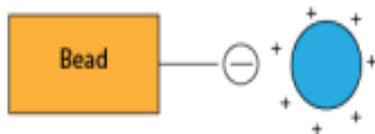
The molecules that bind to the cation exchanger must have a net positive charge, which means that the pH of the solution must be below the protein's isoelectric point (pI) for effective binding to occur. As the salt concentration increases, so does the concentration of Na⁺ (cation) and Cl⁻ (anion), and beyond a certain threshold, the positively charged proteins are displaced and exchanged with the cations in the solution. This process, in which proteins are selectively bound and eluted based on their charge, is known as cation exchange chromatography (figure 38).

Carboxymethyl (CM)	-O-CH ₂ -COO
Sulphopropyl (SP)	-O-CH ₂ -CH ₂ -CH ₂ SO ₃ -
Methyl sulphonate (S)	-O-CH ₂ SO ₃ -

Step 1 Na^+ is attached to negatively charged cation exchanger



Step 2 Positively charged Protein exchanges with Na^+ and binds to cations exchanger. Negatively charged Protein does not bind.



Step 3 A competing Na^+ (NaCl gradient) elutes the Protein. Protein with less positive charge at given pH shall be eluted at lower NaCl concentration while Protein with higher positive charge get eluted at higher concentration at NaCl .

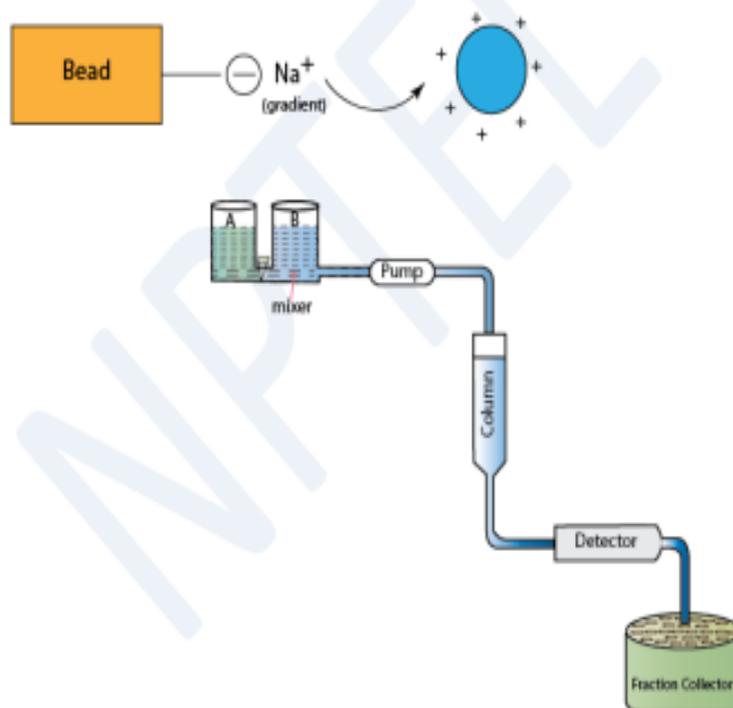


Figure 38: Various steps of cation exchange chromatography and experimental set-up

IV.2.1.2.4. Anion Exchange Chromatography

Anion-exchange chromatography (figure 39) is a technique that separates substances based on their charge using a resin with positively charged groups, such as diethyl-aminoethyl (DEAE). In this process, the resin is initially coated with positively charged counter-ions (cations), and the anion exchange resin binds to negatively charged molecules, displacing the counter-ions. This method is commonly used to purify proteins, amino acids, carbohydrates, and other negatively charged acidic substances at higher pH levels, with the binding strength depending on the substance's negative charge. The specific conditions for anion exchange chromatography, including buffer pH and the choice of counter-ions, can be adjusted based on the protein of interest, the buffer system, and the selected resin. Since ion exchange chromatography relies on electrostatic interactions between the resin and the target molecules, precise control of these factors is essential for effective protein binding to the column resin.

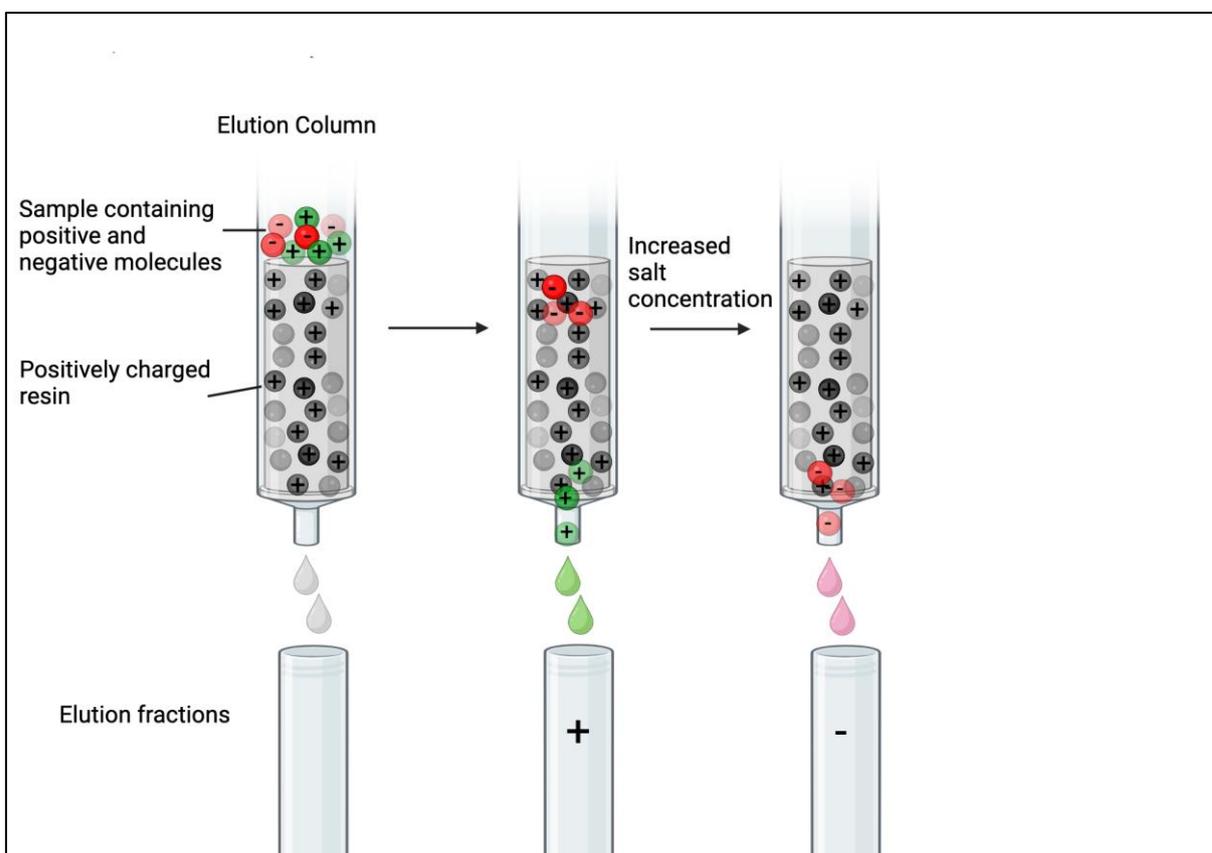


Figure 39: Anion Exchange Chromatography

IV.2.1.3. Size-exclusion chromatography

Exclusion chromatography allows for the separation of molecules through a porous gel based on the size, shape, and molecular weight of the sample to be separated. The stationary phase consists of polysaccharide beads, while the mobile phase is an eluent (such as water or pH buffer) that moves the molecules in the mixture. In this technique, there are no interactions between the liquid mobile phase and the stationary phase. Therefore, the molecules remain in solution throughout their separation.

The basic principle of this technique is simple: the porous beads that make up the gel form a three-dimensional network, and the accessibility of this network is determined by the pore size. The steric hindrance of the molecules and the mesh of the network determine their exclusion. Excluded molecules travel directly between the beads, while others are delayed as they pass through the beads (figure 40). A detection system placed inline detects the passage of different molecules (fractions). The type of support (such as dextran, acrylamide, or mixed support) and the pore diameter, which varies depending on the bead size, determine the range of molecule sizes for which separation is effective: this is the fractionation range.

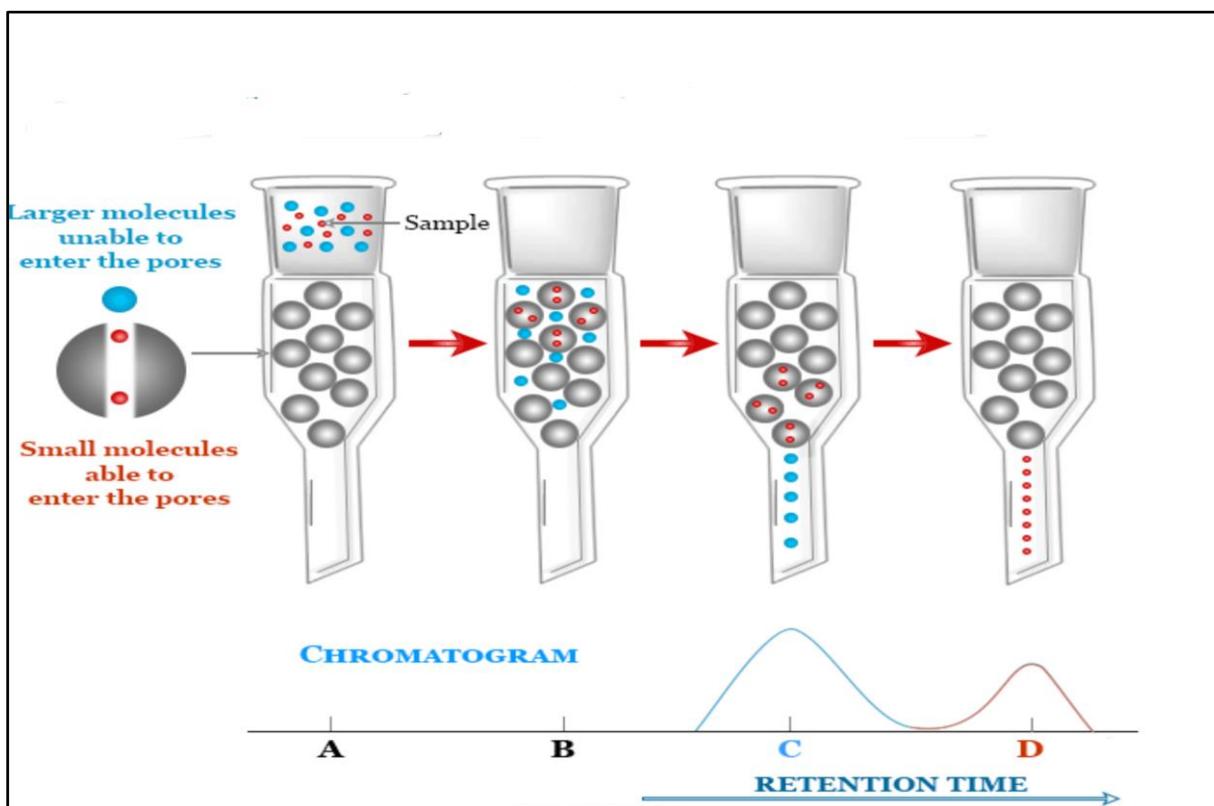


Figure 40: Exclusion Chromatography

IV.2.1.4. Gel electrophoresis

Biomolecules often carry positive or negative electrical charges. When exposed to an electric field, these charged molecules move toward the electrode with the opposite charge due to electrostatic attraction. Electrophoresis is the technique used to separate charged molecules in an electric field. The mobility of each molecule depends on several factors, including its net charge, charge-to-mass ratio, molecular shape, and the temperature, porosity, and viscosity of the medium through which it migrates. This method allows for the high-resolution separation of complex mixtures.

IV. 2.1.4.1. Principle Gel electrophoresis

Electrophoresis is the process by which charged molecules or particles migrate in an electric field when in aqueous solution. The migration speed depends on factors such as the strength of the electric field and the charges on the molecules. As a result, molecules with different charges will form distinct zones during migration (figure 41). To minimize diffusion, electrophoresis is typically performed in an anticonvective medium like a viscous fluid or gel matrix, which also affects the speed of migration based on the size of the molecules. This allows for high-resolution separation of complex mixtures.

When a mixture of electrically charged biomolecules is placed in an electric field, they move toward the electrode with the opposite charge. However, molecules move at different rates depending on their physical characteristics and the experimental conditions. The velocity of a charged molecule in an electric field is given by the equation:

$$v = Eq / f$$

where v is the velocity, E is the electric field strength, q is the net charge of the molecule, and f is the frictional coefficient. The frictional coefficient is influenced by factors such as the molecule's mass, compactness, the viscosity of the buffer, and the porosity of the matrix. The net charge of a molecule is determined by the number of positive and negative charges it carries, which can arise from amino acid side chains or post-translational modifications like phosphorylation or acylation. For DNA, the phosphate group provides a uniform negative charge per nucleotide.

The equation suggests that molecules move faster with higher net charges, stronger electric fields, and lower frictional coefficients (which depend on molecular mass and shape). Molecules with similar charges separate based on differences in their frictional coefficients, while molecules with similar mass/shape may differ significantly in net charge. This allows for

very high-resolution separation, making electrophoresis a powerful technique for fractionating complex mixtures.

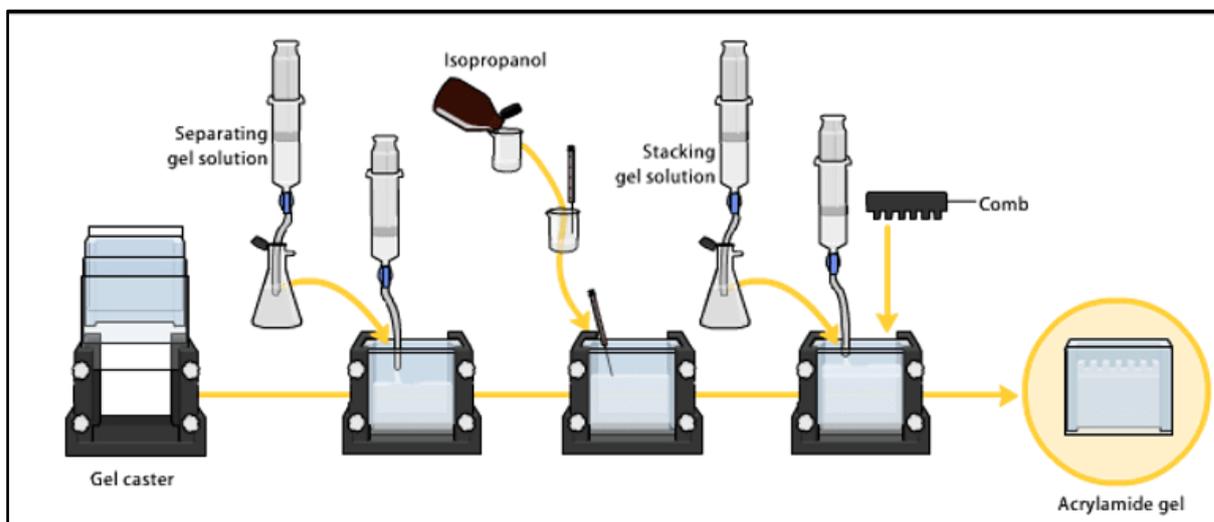


Figure 41 : Gel electrophoresis: Principle and procedure

IV.2.1.4.2. Types Gel electrophoresis

The types of gel electrophoresis include:

1. Agarose Gel Electrophoresis: Primarily used for the separation of nucleic acids (DNA and RNA), this method uses agarose gel, a porous matrix that separates molecules based on their size.
2. Polyacrylamide Gel Electrophoresis (PAGE): Commonly used for the separation of proteins and smaller nucleic acid fragments, PAGE offers higher resolution than agarose gel electrophoresis. It is used in techniques like SDS-PAGE for proteins and native PAGE for proteins in their natural form.
3. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE): A variation of PAGE that separates proteins based on their molecular weight. SDS denatures proteins and gives them a uniform negative charge.
4. Isoelectric Focusing (IEF): Separates proteins based on their isoelectric point (pI), which is the pH at which a protein has no net charge. It is often combined with other methods, such as 2D-PAGE, for more detailed analysis.

5. **2D Gel Electrophoresis:** Combines isoelectric focusing (IEF) in the first dimension and SDS-PAGE in the second dimension, allowing for high-resolution separation of complex mixtures, particularly in proteomics.
6. **Capillary Electrophoresis (CE):** A high-precision method that uses small capillaries filled with a gel or buffer solution for the analysis of small molecules, peptides, and nucleic acids.
7. **Pulsed-Field Gel Electrophoresis (PFGE):** A specialized technique for separating large DNA molecules, such as bacterial chromosomes, by applying an alternating electric field in different directions, enabling the separation of very large DNA fragments.

These types of gel electrophoresis are used for different types of biomolecule analysis, depending on the size, charge, and characteristics of the molecules being studied.

IV.3. Sterilization and Decontamination

Sterilization and decontamination are vital processes used to eliminate or reduce harmful microorganisms such as bacteria, viruses, fungi, and other pathogens. These procedures are essential for ensuring safety and preventing contamination across various industries, including healthcare, pharmaceuticals, food production, and environmental protection.

Sterilization

Sterilization involves the complete destruction or removal of all microbial life, including tough bacterial spores. It is a rigorous process designed to ensure that no viable microorganisms remain on surfaces, tools, or products. Sterilization is especially critical in medical and pharmaceutical environments, where maintaining aseptic conditions is essential. Common methods of sterilization include:

- **Filtration:** This technique removes microorganisms by passing liquids or gases through a filter. It is frequently used in pharmaceuticals to sterilize heat-sensitive liquids and in water purification systems to remove microbial contaminants.
- **Heat Sterilization:** This method includes autoclaving (steam under pressure) and dry heat sterilization, both of which are effective for sterilizing medical instruments, glassware, and other heat-resistant materials. Autoclaving is widely used in healthcare settings for sterilizing surgical tools and equipment.
- **UV Light:** Ultraviolet (UV) light is a non-thermal sterilization method that inactivates microorganisms by damaging their DNA. It is used in water purification systems,

laboratories, and pharmaceutical production to eliminate microbial contamination in air, surfaces, and liquids.

Decontamination

Decontamination refers to the process of cleaning or disinfecting surfaces or environments to reduce or eliminate harmful microorganisms to a safe level. While it is generally less intensive than sterilization, decontamination is still crucial for maintaining hygiene in various industries.

The main methods of decontamination include:

- **Cleaning:** This is the process of removing dirt, debris, and microorganisms from surfaces or objects, often as the first step before disinfection or sterilization.
- **Disinfection:** A procedure that reduces the number of viable microorganisms to a level deemed safe. It usually involves chemicals like chlorine-based disinfectants, alcohol, or iodine.
- **Antisepsis:** A specialized form of disinfection applied to living tissues (such as skin) to prevent infection. It is commonly used in medical procedures and healthcare environments.

Both sterilization and decontamination are essential for ensuring safety and preventing the spread of harmful microorganisms across different settings, from healthcare facilities to food production environments.

IV. 3.1. Applications

Sterilization and decontamination methods play a crucial role in various industries to ensure the safety and purity of products and environments. These processes, including filtration and UV light, are essential for microbial purification in water, pharmaceuticals, and laboratory settings. In water treatment, both sterilization and decontamination methods, such as filtration and UV light, are utilized to remove harmful pathogens, ensuring that water is safe for drinking and industrial use. In the pharmaceutical industry, sterilization techniques like heat sterilization and filtration are vital to ensure that drugs, biologics, and medical devices are free from microbial contamination. UV light is also employed in pharmaceutical production to maintain aseptic conditions in cleanrooms. Similarly, in laboratory settings, sterilization methods like filtration and UV light are used to create contamination-free environments, crucial for experiments involving microorganisms or sensitive samples. These processes are critical for maintaining safety and quality across multiple sectors.

IV.4. Biodegradable and Green Purification Methods

Biodegradable and eco-friendly purification methods emphasize environmentally conscious approaches to removing impurities, contaminants, or toxins from substances like water, air, and chemicals. These techniques are designed to avoid harming ecosystems and minimize the use of hazardous chemicals. They focus on using sustainable materials, enhancing energy efficiency, and reducing environmental impact, all while ensuring the purification process remains effective.

IV.4. 1. Principles of Biodegradable and Green Purification Methods

The fundamental concept of green purification is to reduce the reliance on harmful substances and lower energy usage, while ensuring effective results. This can be accomplished by using natural, biodegradable materials, renewable energy sources, and environmentally conscious techniques. The objective is to ensure that purification processes are free from pollution and waste, fostering sustainability and non-toxicity by utilizing renewable resources.

IV.4. 2. Types of Biodegradable and Green Purification Methods

There are several innovative methods in the field of purification that focus on sustainability and environmental responsibility. These biodegradable and eco-friendly techniques not only reduce the use of harmful chemicals but also minimize energy consumption, making them more sustainable alternatives to traditional methods.

Among these methods, we can mention:

- **Bioremediation:** This method utilizes living organisms such as bacteria, fungi, or plants to break down or transform harmful pollutants into less toxic substances. It is commonly used for cleaning contaminated environments, including soil, water, and air, such as the degradation of hydrocarbons in oil spills in oceans.
- **Phytoremediation:** This technique involves the use of plants to remove, degrade, or stabilize environmental contaminants. Plants can absorb heavy metals from the soil, detoxify organic compounds in water, or enhance air quality by capturing pollutants like carbon dioxide and volatile organic compounds (VOCs).

- **Green Solvents:** Green solvents, including supercritical CO₂ and bio-based solvents derived from renewable resources, are gradually replacing traditional toxic solvents in chemical purification processes. These solvents are biodegradable, non-toxic, and generally require less energy to apply, offering a more sustainable alternative.
- **Natural Coagulation and Flocculation:** In water treatment, natural coagulants like moringa seeds or chitosan are being used to replace synthetic chemicals like alum. These biodegradable materials effectively remove impurities from water, providing eco-friendly solutions, especially in rural and developing regions.
- **Electrocoagulation:** This process employs electrical currents to remove contaminants from water, reducing the reliance on chemical additives. It is particularly useful for treating industrial effluents and municipal wastewater, providing an environmentally friendly method for water purification.

IV.4. 3. Applications of Biodegradable and Green Purification Methods

Green purification methods are increasingly being applied across various sectors to address environmental challenges and improve sustainability (figure 42). In water purification, techniques such as bioremediation, phytoremediation, and the use of natural coagulants are gaining traction. For example, moringa seeds are used in rural areas as a natural, affordable, and biodegradable solution to remove suspended particles and microorganisms, offering an eco-friendly alternative for clean water access. Similarly, air purification benefits from phytoremediation, where plants absorb carbon dioxide, VOCs, and other pollutants, and bioremediation, which aids in degrading harmful airborne compounds, thereby improving urban air quality and mitigating the environmental impact of industrial emissions.

In wastewater treatment, biodegradable and green purification methods, such as electrocoagulation, bioremediation, and green solvents, are increasingly used to treat industrial wastewater, municipal effluents, and food processing waste. These methods help reduce pollution, support water recycling, and promote more sustainable practices in wastewater management. Furthermore, in the chemical and pharmaceutical industries, green solvents and enzymatic processes are transforming production methods by replacing toxic chemicals with renewable, non-toxic alternatives. This shift results in safer production processes, greener

formulations, and reduced waste, ultimately contributing to a more sustainable and environmentally responsible approach to industrial purification across multiple sectors.

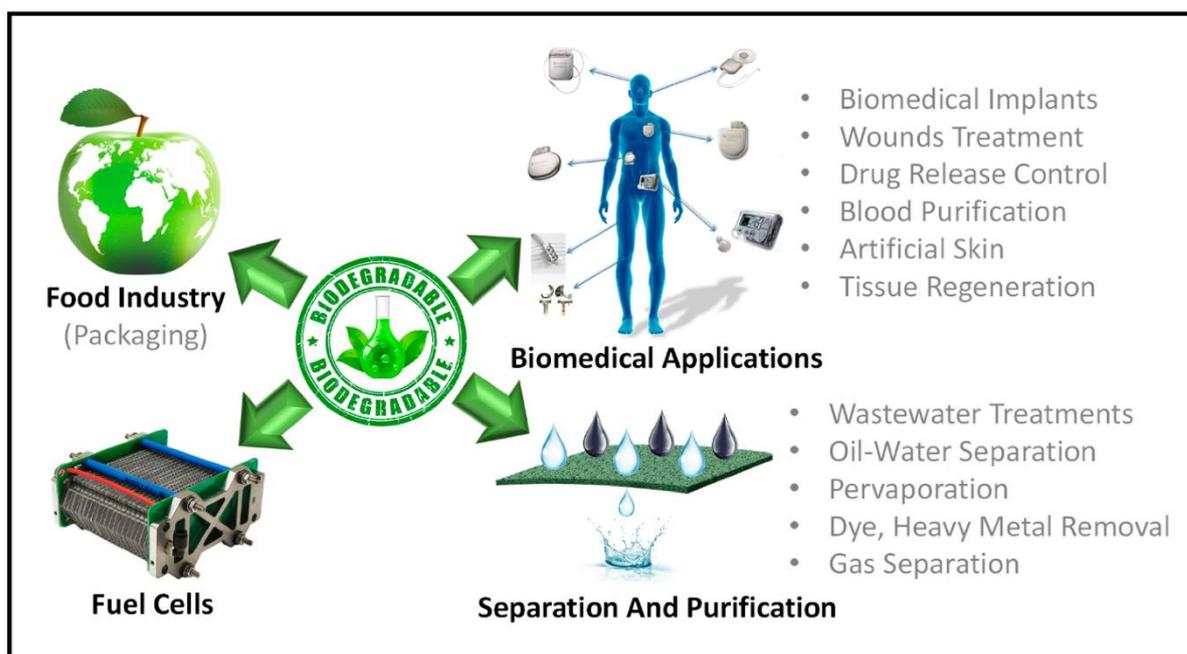


Figure 42 : Applications of biodegradable polymers-based membranes.

IV.5. Emerging Methods Focusing on Sustainability

Emerging green purification methods are increasingly focused on sustainable alternatives that minimize environmental impact, particularly through the use of natural reagents, enzymes, and solvent-free technologies. Enzymatic processes, widely adopted in wastewater treatment, food processing, and pharmaceutical manufacturing, provide targeted purification by utilizing specific enzymes to break down contaminants or process bio-waste efficiently. These enzymes offer a more environmentally friendly solution compared to traditional chemical methods, as they operate under mild conditions and do not produce hazardous byproducts, making them ideal for applications where precision and sustainability are key.

In addition to enzymatic processes, advancements in solvent-free technologies are revolutionizing purification methods by reducing or eliminating the need for toxic chemical solvents. Techniques such as supercritical fluid extraction (SFE) and membrane filtration are gaining traction across industries like pharmaceuticals, food processing, and chemical manufacturing. Supercritical fluid extraction, for example, uses supercritical CO₂, a non-toxic, environmentally benign solvent, to extract valuable compounds from raw materials without the use of traditional organic solvents. Membrane filtration, on the other hand, relies on physical barriers to separate contaminants, offering an energy-efficient and chemical-free method of

purification. Both of these technologies maintain high purification efficiency while being cost-effective and eco-friendly, further supporting the shift towards greener, more sustainable practices in various industrial sectors.

IV.6. Applications of Purification Methods

Purification methods are widely applied across various industries to ensure the removal of impurities and contaminants, achieving high levels of purity in materials, products, and environments. In the pharmaceutical industry, purification is critical for producing drugs with high purity and efficacy, ensuring that unwanted compounds or impurities are removed during manufacturing. Techniques such as chromatography, distillation, and filtration are commonly used to isolate bioactive compounds, synthesize chemicals, and purify medications. In chemical manufacturing, purification ensures the production of high-quality chemicals and materials, such as polymers, solvents, and reagents, with minimal contamination that could affect performance or safety. Water treatment relies on purification methods like reverse osmosis, filtration, and bioremediation to remove pollutants, pathogens, and toxins, ensuring safe drinking water and clean wastewater. In the food industry, distillation, filtration, and adsorption are employed to purify ingredients, preserve food quality, and remove harmful substances, such as pesticides and heavy metals, from food products. Additionally, biotechnology benefits from purification methods like affinity chromatography and gel electrophoresis to isolate proteins, enzymes, and nucleic acids, which are essential for research and the production of therapeutics. Across these sectors, purification not only enhances product quality and safety but also plays a key role in environmental protection by ensuring that waste products are appropriately treated and pollutants are removed.

IV.6.1. Pharmaceutical Industry

Purification plays a vital role in the pharmaceutical industry by ensuring the production of safe, effective, and high-quality drugs. The process involves removing impurities, by-products, and contaminants from raw materials, intermediate compounds, and final drug formulations. Common techniques, such as chromatography (including affinity, reverse-phase, and ion-exchange chromatography), distillation, and filtration, are widely used to isolate and purify bioactive compounds. Chromatography, for example, is particularly effective for separating complex mixtures based on differences in size, charge, or affinity for the stationary phase. Distillation is employed to purify solvents or separate volatile components, while filtration is often used for sterilizing and removing particulate matter from pharmaceutical products.

Purification ensures that pharmaceutical products meet strict regulatory standards, such as Good Manufacturing Practices (GMP), which require the removal of impurities that could compromise drug safety and efficacy. Biopharmaceuticals, including monoclonal antibodies, vaccines, and recombinant proteins, necessitate specialized purification techniques to isolate these biomolecules from complex mixtures. Methods like ultrafiltration, size-exclusion chromatography, and affinity chromatography are commonly applied in this context.

In the production of active pharmaceutical ingredients (APIs), purification is essential for achieving the required purity levels, ensuring therapeutic efficacy, and minimizing the risk of adverse reactions (figure 43). Furthermore, in drug development, purification methods are crucial for drug discovery and the synthesis of pure compounds for clinical trials. As the pharmaceutical industry continues to innovate, there is an ongoing focus on enhancing purification techniques to improve manufacturing processes, increase efficiency, and reduce costs, with an emphasis on sustainability.

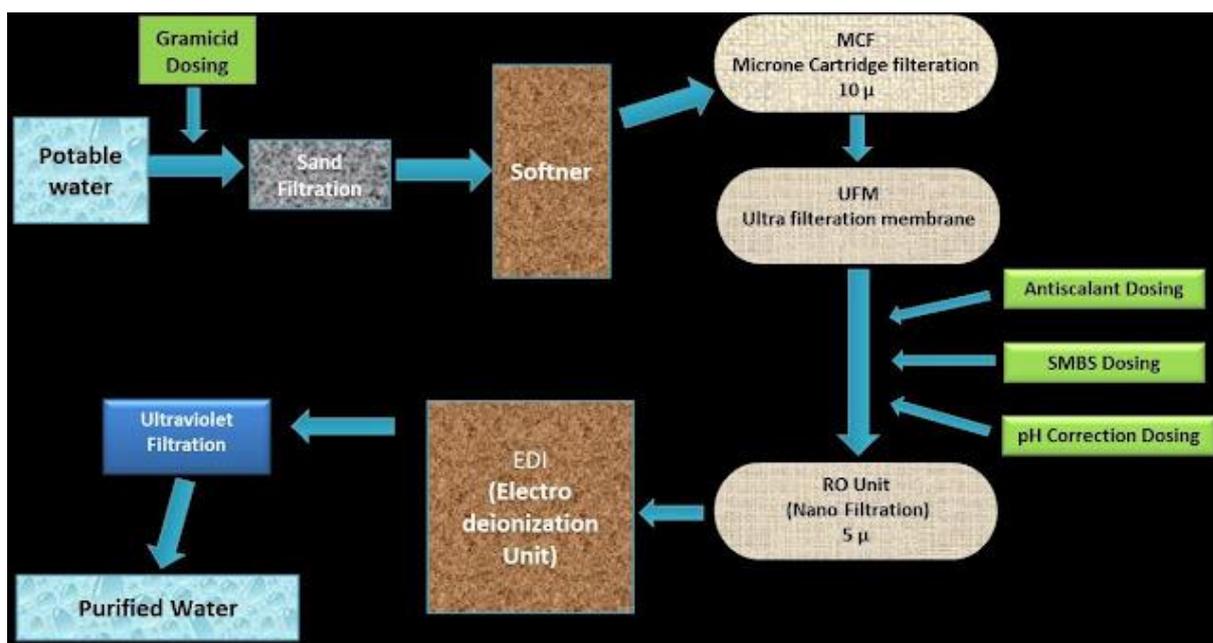


Figure 43: Operational Flow of Pharmaceutical Purified water generation system

IV.6.2. Food and Beverage Industry

In the food and beverage industry, purification methods are essential for ensuring product safety, quality, and consistency. These techniques are used to remove contaminants, undesirable compounds, and impurities from raw materials, ingredients, water, and final products, ensuring compliance with health regulations and industry standards. Purification processes help maintain the desired flavor, texture, and nutritional value of food and beverages, while also extending

shelf life. Filtration is one of the most commonly employed methods, particularly effective in removing solid particles, sediments, and microbial contaminants from liquids such as water, juices, and beverages. Membrane filtration techniques, including reverse osmosis, are widely used to purify water and concentrate ingredients, while microfiltration and ultrafiltration help clarify liquids and separate components based on size.

In addition to filtration, distillation plays a significant role in alcoholic beverage production, such as whiskey and vodka, where it is used to separate alcohol from the fermentation mixture. For removing specific unwanted components like toxins or pesticides, techniques such as solvent extraction and adsorption are often applied. Centrifugation is commonly used in dairy processing to separate cream from milk, while chromatography is increasingly used to purify bioactive compounds, flavors, and natural colorants, enhancing product quality. Ion exchange is also employed to remove impurities from beverages, such as softening water for brewing or clarifying juices.

Purification methods are also critical for food preservation. Techniques like pasteurization and sterilization, including heat sterilization and UV light exposure, are used to eliminate harmful microorganisms, ensuring food safety during storage and transport. Additionally, the removal of contaminants such as heavy metals, preservatives, and allergens is increasingly important to meet the demand for clean-label products and cater to health-conscious consumers. These purification methods work together to ensure that food and beverages are safe, high-quality, and meet the evolving expectations of consumers.

IV.6.3. Environmental Applications

Purification methods are essential for environmental protection, addressing pollution and safeguarding ecosystems through wastewater treatment, air purification, and soil remediation. These techniques help reduce contaminants and ensure the health and sustainability of natural resources.

In wastewater treatment, purification processes such as filtration, membrane filtration (e.g., reverse osmosis and ultrafiltration), and biological treatments (e.g., activated sludge systems) are used to remove pollutants from industrial, municipal, and agricultural wastewater. These methods effectively eliminate harmful microorganisms, chemicals, nutrients (like nitrogen and phosphorus), and heavy metals, ensuring that the water is safe for release into the environment or can be reused in various industries. More advanced techniques, including membrane

bioreactors and chemical precipitation, are employed to remove complex contaminants more efficiently.

For air purification, technologies like electrostatic precipitators, scrubbers, and activated carbon filtration capture particulate matter, volatile organic compounds (VOCs), and gases such as sulfur dioxide and nitrogen oxides from industrial emissions. These methods are critical in reducing air pollution, improving air quality, and mitigating the harmful effects of industrial activities, vehicle emissions, and power plants on both public health and the environment.

Soil remediation is equally important, focusing on removing or neutralizing pollutants in contaminated soil, often resulting from industrial activities, agricultural runoff, or waste disposal. Common techniques such as chemical treatment, bioremediation, soil washing, and phytoremediation help detoxify harmful substances like heavy metals, pesticides, and hydrocarbons, restoring soil health for agricultural and ecological purposes.

Emerging green purification technologies, including the use of biological agents, natural reagents, and enzyme-based processes, are gaining prominence in these environmental applications. These more sustainable methods reduce the reliance on harsh chemicals and energy-intensive processes, helping to minimize the overall environmental impact while ensuring effective purification and protection of natural resources.

IV.6.4. Industrial Applications

Purification methods are essential across a wide range of industries to ensure that raw materials, intermediate products, and final goods meet the necessary purity standards for both functionality and safety. In industries such as pharmaceuticals, chemical manufacturing, petrochemical refining, food processing, metal purification, and biotechnology, purification plays a critical role in quality control, regulatory compliance, and process efficiency.

In the pharmaceutical sector, purification techniques are vital for isolating and purifying active pharmaceutical ingredients (APIs), excipients, and other compounds. Methods like chromatography, distillation, precipitation, and membrane filtration are used to eliminate impurities, contaminants, and by-products from drug substances, ensuring the production of safe, high-quality medicines. Sterilization methods, including autoclaving, UV light, and filtration, are also essential for ensuring microbial safety in pharmaceutical products like injectables, vaccines, and biologics.

Similarly, in chemical manufacturing and petrochemical refining, purification is key to producing high-purity chemicals, solvents, and fuels. Techniques such as distillation, solvent extraction, and ion exchange help separate desired products from impurities, enhancing quality

and minimizing contamination. For example, fractional distillation is commonly used to separate crude oil components into valuable products like gasoline and diesel, while solvent extraction recovers valuable metals or rare earth elements from ores and waste streams. Other techniques, such as catalyst regeneration and adsorption, improve process efficiency by removing unwanted by-products.

In metal purification, techniques like electrolytic refining, solvent extraction, and precipitation are crucial for purifying metals like copper, gold, and silver. These methods remove impurities such as oxides and sulfur, ensuring the high purity of metals used in critical applications like electronics and automotive components.

The food and beverage industry also heavily relies on purification methods to maintain product safety and quality. Filtration, reverse osmosis, and distillation are used to purify ingredients like water, oils, juices, and milk. Membrane filtration is particularly important in dairy and beverage industries for removing bacteria, pathogens, and excess salts. In food processing, adsorption and decolorization techniques help eliminate undesirable flavors, colors, and contaminants from oils, juices, and sweeteners, while the purification of food additives, preservatives, and nutrients ensures product consistency and safety.

In biotechnology, purification methods are essential for the production of biopharmaceuticals, enzymes, and biofuels. Techniques such as affinity chromatography, gel electrophoresis, and ion-exchange chromatography isolate proteins, enzymes, nucleic acids, and other bioproducts from complex biological mixtures. These methods are especially important for the purification of therapeutic proteins, monoclonal antibodies, and vaccines, which require extremely high levels of purity. Additionally, membrane filtration and centrifugation are critical in large-scale bioprocessing, enabling the separation of cell cultures, harvests, and fermentation broths to produce high-purity bioproducts.

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