Exploring the History and Advances of Chromatography: Separating Mixtures for Scientific Progress

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Abstract
Chromatography is a separation method that divides mixture components according to their physical and chemical characteristics. Chromatography was first discovered in the early 19th century, and Mikhail Tswett, a Russian botanist, made the first significant advancement in the field of chromatography in 1903. Chromatography has played a significant role in the advancement of many fields, including biochemistry, pharmaceuticals, and environmental science. There are various chromatographic techniques, such as gas chromatography and paper chromatography. Ultra-high-performance liquid chromatography (UHPLC) and two-dimensional chromatography are recent developments in chromatography technology. Chromatography is a vital technique in scientific research that enables the separation, purification, and identification of components in complex mixtures such as mixtures of amino acids and other biomolecules.

Keywords: Chromatography, Paper Chromatography, HPLC

Introduction
Chromatography is a separation technique that divides mixture components according to their physical and chemical characteristics (Kapoor et al., 2011). The technique involves a mobile phase that flows through a stationary phase, which can be solid or liquid, and the components of the mixture interact differently with it, leading to their separation (Skinner et al., 2015). It is widely used in various fields, including chemistry, biochemistry, pharmaceuticals, food science,

and environmental science, to analyze and identify components of complex mixtures (Borges et al., 2018).

Chromatography is an important tool used in scientific research for the separation, purification, and identification of compounds from complex mixtures (Skinner et al., 2015). The technique is commonly used in analytical chemistry to quantify the amount of a particular compound in a sample (Borges et al., 2018). It is also used in the development of new drugs and other products, as well as in the analysis of food and environmental samples (Kapoor et al., 2011).

Chromatography is a technique used in biochemistry to distinguish between and separate biomolecules like proteins, nucleic acids, and carbohydrates (Skinner et al., 2015). The purification and separation of drug molecules using chromatography is common in the pharmaceutical industry (Borges et al., 2018). It is also an essential tool in forensic science, where it is used to identify and quantify drug metabolites, toxins, and other chemical substances in bodily fluids (Borges et al., 2018).

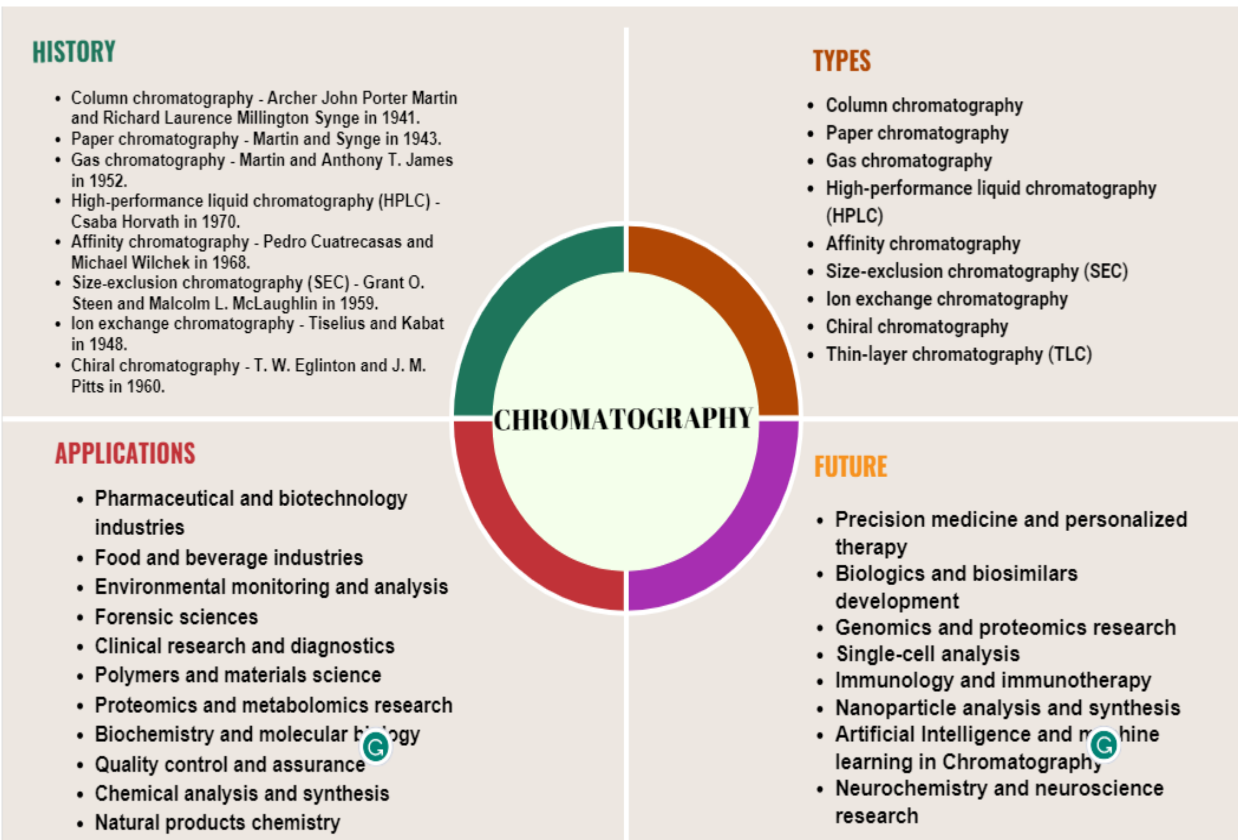

Figure 1: Graphical Abstract (Created on Chemix)

History of Chromatography

Chromatography's origins can be traced to the early 19th century when the process was first discovered. In 1879, Friedrich Goppelsroeder used chromatography to separate and identify the constituents of plant extracts (Skinner et al., 2015). In 1903, Mikhail Tswett made the first significant breakthrough in the field of chromatography by separating the pigments in plants using a column packed with calcium carbonate. The technique was named chromatography from the Greek words "chroma" meaning color, and "graphein" meaning to write (Kapoor et al., 2011).

Paper chromatography and gas chromatography were developed in the 1940s and 1950s, respectively, which greatly improved the efficiency and accuracy of the technique. In 1944, Archer Martin and Richard Synge invented paper chromatography, which involved the use of filter paper to separate amino acids (Borges et al., 2018). Gas chromatography was invented around the same time, with the first gas chromatograph being built in 1947 by James and Martin (Skinner et al., 2015).

In the 1960s, high-performance liquid chromatography (HPLC) was developed, which allowed for faster and more accurate separations than traditional liquid chromatography (Kapoor et al., 2011). In the 1970s, new types of chromatography were developed, including size-exclusion chromatography and ion chromatography (Borges et al., 2018). Significant improvements in chromatography technology have been made recently, including the development of two-dimensional chromatography and ultra-high-performance liquid chromatography (UHPLC) (Skinner et al., 2015).

In summary, chromatography is an essential technique in scientific research that enables the separation, purification, and identification of components in complex mixtures. Its applications are broad and diverse, making it an indispensable tool in various fields. There have been significant advancements in chromatography technology since its discovery, leading to improved efficiency and accuracy in separation and identification of components.

Types of Chromatography

Paper chromatography:

Paper chromatography is a type of chromatography that involves the use of filter paper as the stationary phase (Pavia et al., 2010). It is a popular method for separating and identifying mixture components because it is simple and affordable. In paper chromatography, a tiny amount of the sample is spotted on the paper before being submerged in a solvent for development. Based on their affinity for the stationary and mobile phases, the various sample components are separated as the solvent moves up the paper. The separated components can be visualized by a variety of techniques, such as staining or fluorescence.

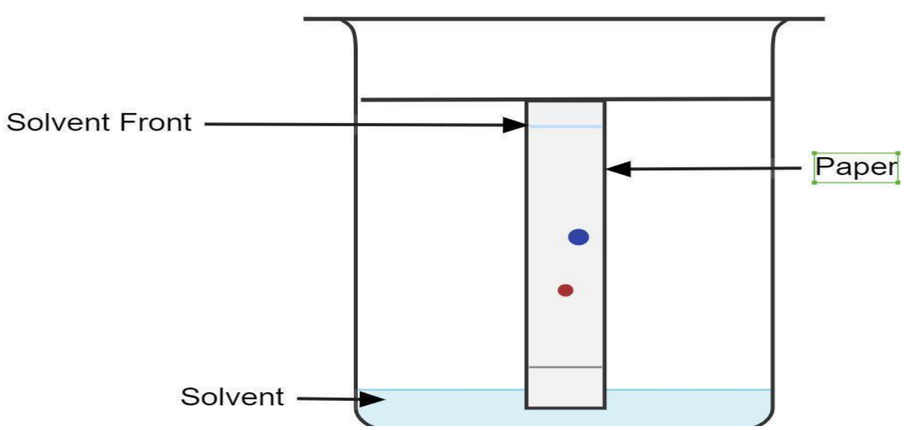

Figure 2: Setup for Paper Chromatography (Created on Chemix.org)

Gas chromatography:

Gas chromatography is a chromatographic method that separates components on the basis of their ability to partition between a stationary phase and a mobile gas phase. In gas chromatography, a small amount of the sample is injected into the column, which is packed with a stationary phase that is typically coated with a liquid (Skell, 2016). A carrier gas, such as helium or nitrogen, is then used to move the sample through the column. According to their affinity for the stationary and mobile phases, the components are separated as they move through the column and interact with the stationary phase. Several methods, including thermal conductivity and mass spectrometry, are used to find the separated components.

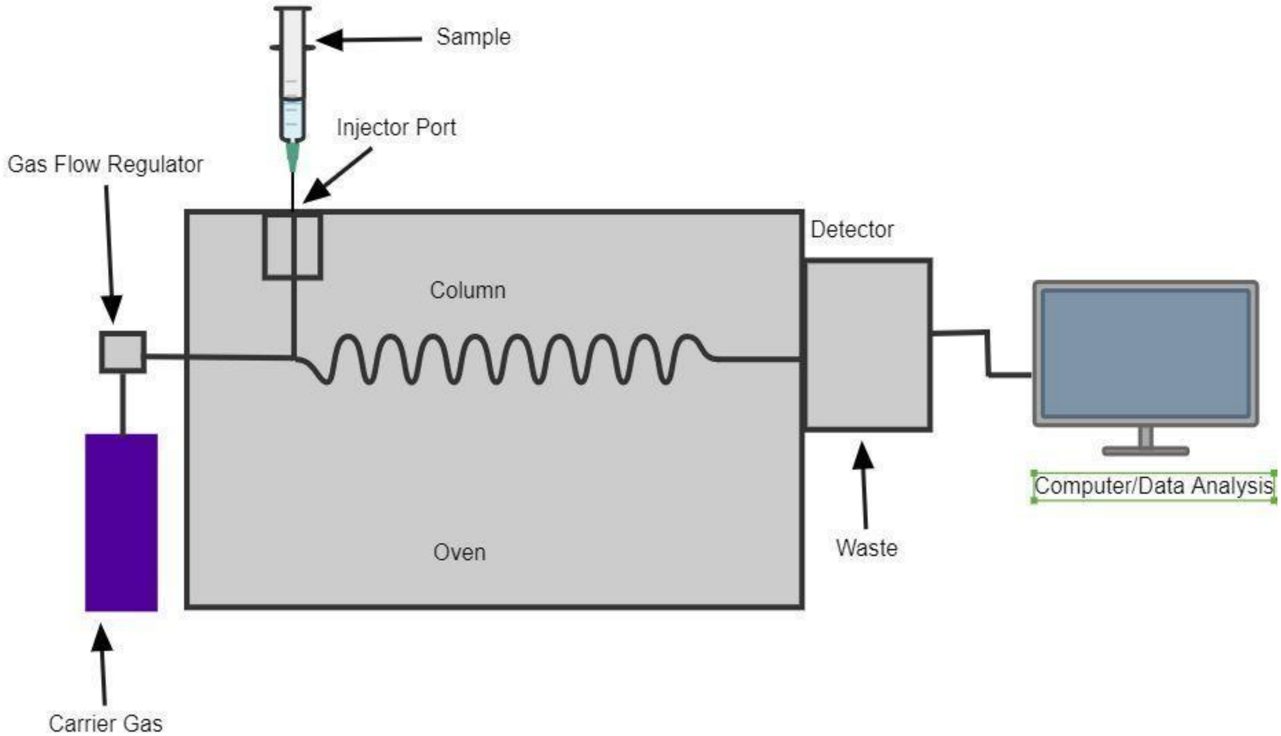

Figure 3: Schematic Diagram of Gas Chromatography Setup (Created on Chemix)

Liquid chromatography:

Liquid chromatography is a chromatographic technique that separates components on the basis of their affinity for a stationary phase and a mobile liquid phase (Majors, 2011). The sample is injected into a column that is lined with a stationary phase, such as silica gel or a polymer, in liquid chromatography. The sample is then passed through the column using the mobile phase, which is typically a solvent. Components are separated based on their affinity for the stationary and mobile phases as they move through the column, interacting with the stationary phase. Several methods, including UV-visible spectroscopy and mass spectrometry, are used to identify the separated components.

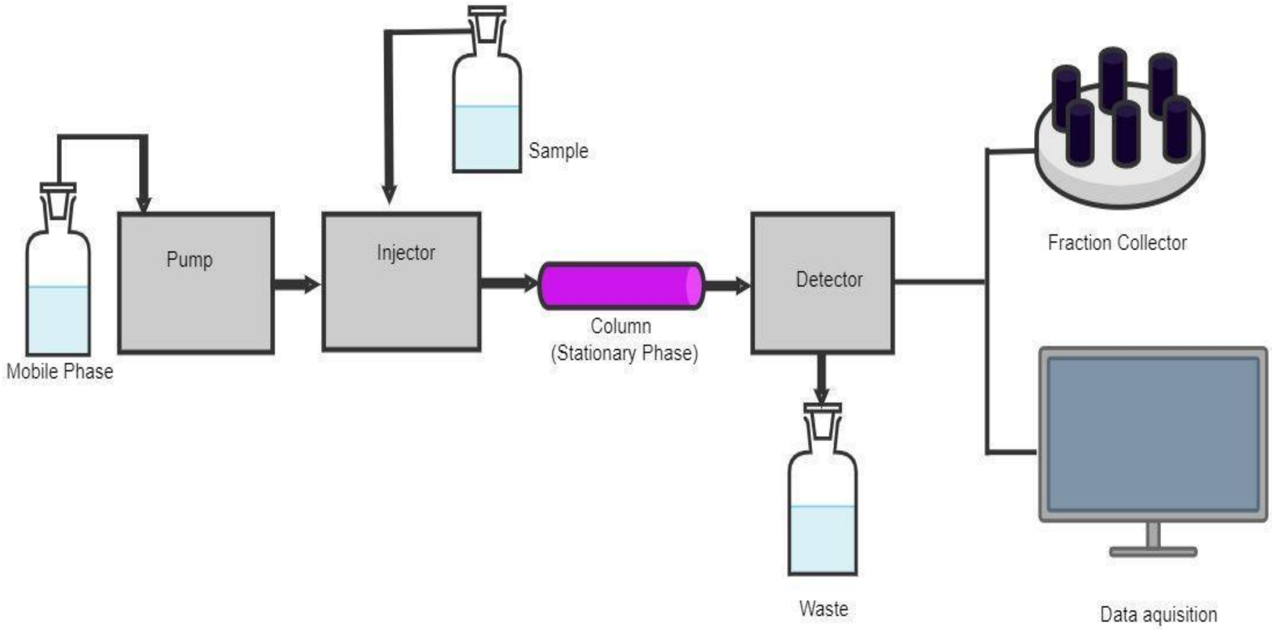

Figure 4: Schematic Diagram of Liquid Chromatography Setup (Created on Chemix)

Ion chromatography:
Ion chromatography is a chromatographic technique that separates components on the basis of their charge (El Rassi, 2017). In ion chromatography, the stationary phase is typically a resin that contains charged groups, such as sulfonic acid or quaternary ammonium. The sample is injected into the column, and a mobile phase, which is typically an electrolyte solution, is used to move the sample through the column. The components interact with the stationary phase as they move through the column and are divided according to their charge. Several methods, including conductivity or UV-visible spectroscopy, are used to find the separated components.

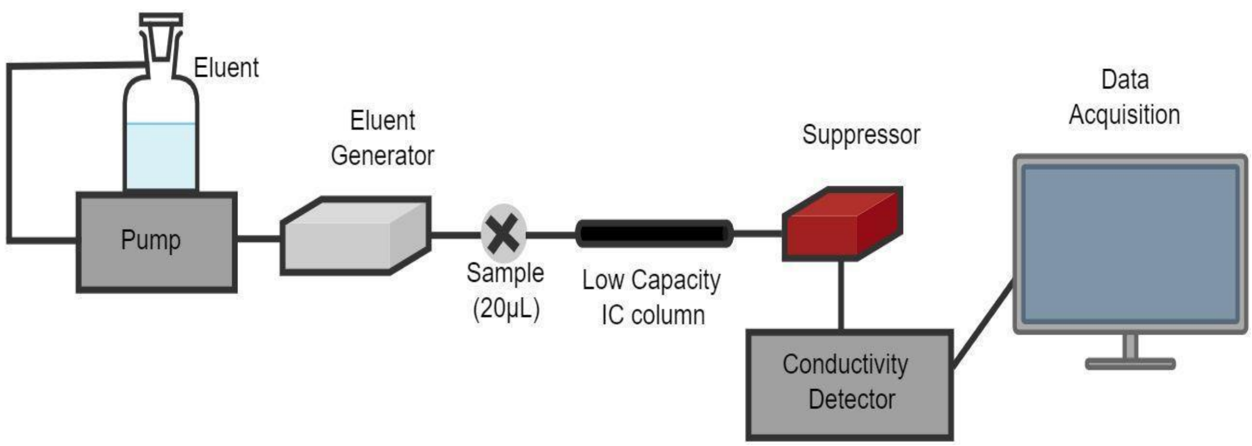

Figure 5: Schematic diagram of Ion Chromatography Setup (Created on Chemix)

High-performance liquid chromatography (HPLC):
In high-performance liquid chromatography (HPLC), the mobile phase is pumped through the column at high pressure (Majors, 2011). HPLC is a quick and effective method that is frequently

used to separate and identify mixture components. Silica gel, polymer beads, or protein-based substances are just a few examples of the different stationary phases that can be used with HPLC. Several methods, including UV-visible spectroscopy and mass spectrometry, are used to identify the separated components.

Size-exclusion chromatography:
A type of liquid chromatography called size-exclusion chromatography separates components according to their sizes. In size-exclusion chromatography, the stationary phase is typically a polymer-based resin with pores of a specific size (Volk, 2011). The injection of the sample into the column is followed by the movement of the sample through the column by the mobile phase. The components are partitioned according to size as they pass through the column. Larger components are excluded from the pores and elute from the column first, while smaller components can enter the pores and are retained longer. Size-exclusion chromatography is commonly used to purify proteins and other biomolecules.

Affinity chromatography:
Affinity chromatography is a type of liquid chromatography in which biomolecules are separated based on their interactions with a ligand immobilized on a stationary phase.(Kohoutova & Brabcova, 2019). The ligand can be a protein, nucleic acid, or small molecule that selectively binds to the target biomolecule, such as an enzyme, antibody, or receptor. The target biomolecule is retained by the affinity matrix as the sample is passed through the column, but the undesirable molecules pass through unaffected. The target biomolecule is then eluted by changing the conditions, such as the pH, salt concentration, or temperature, to disrupt the binding interaction. Affinity chromatography is widely used in biochemistry, biotechnology, and pharmaceutical research to isolate and purify proteins and other biomolecules for further study or therapeutic use.

Chiral chromatography:
Chiral chromatography is a type of liquid or gas chromatography that separates enantiomers, which are mirror images of each other (Zhang et al., 2019). Except for their capacity to rotate plane-polarized light in opposite directions, enantiomers are identical in terms of their physical and chemical characteristics. In chiral chromatography, the stationary phase is typically a chiral selector, such as a cyclodextrin or a protein-based resin, that has a specific affinity for one enantiomer over the other. The sample is injected into the column, and the enantiomers are separated based on their differential interactions with the stationary phase. Chiral chromatography is used in various fields, including pharmaceuticals, agrochemicals, flavors, and fragrances, where enantiomeric purity is critical for biological activity, safety, or sensory properties.

Thin-layer chromatography:
According to their differential adsorption and migration on a thin layer of stationary phase coated on a solid support, such as glass or plastic, small molecules are separated using thin-layer chromatography (TLC), a type of planar chromatography (Majors, 2011). The stationary phase can be made of silica gel, alumina, cellulose, or other materials with varying polarities and functionalities. The plate is loaded into a developing chamber with a solvent or solvent mixture that moves up the plate by capillary action after the sample has been spotted on the stationary phase. The components of the sample separate and take the form of distinct spots or bands as the solvent moves over the stationary phase. The separation is visualized by treating the plate with a chemical reagent or UV light that reveals the presence and location of the components. TLC is a

simple and inexpensive technique that is widely used in analytical chemistry, biochemistry, and forensic science for qualitative and quantitative analysis, identification, and purification of small molecules.

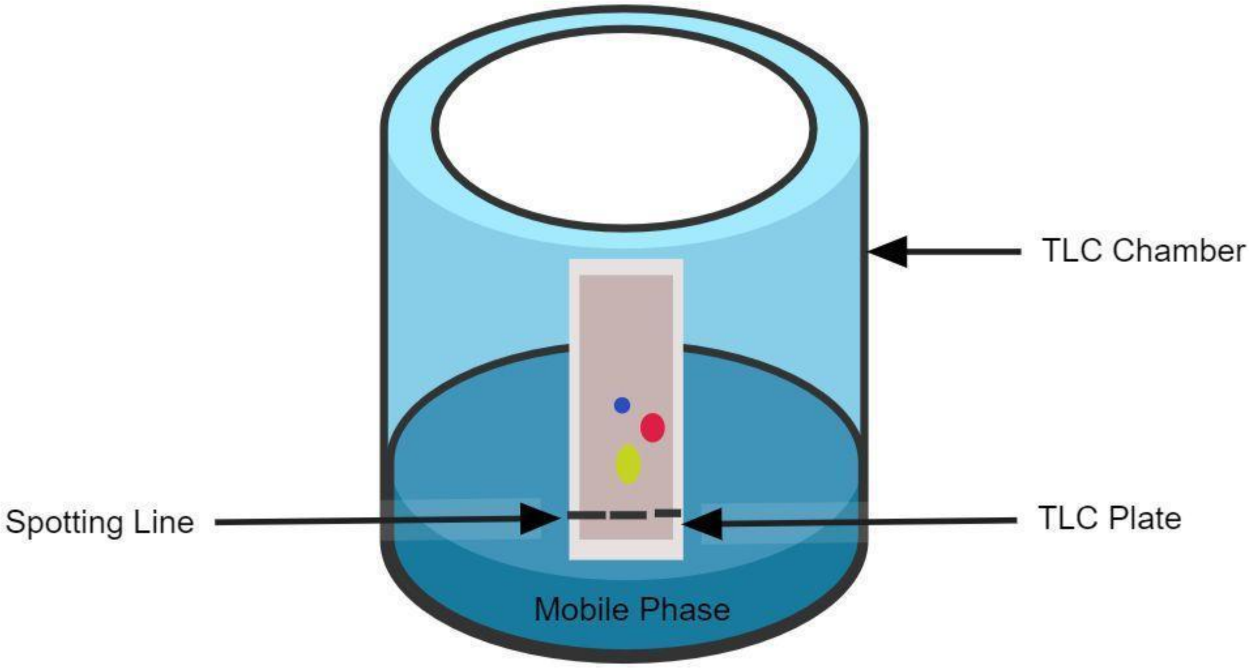

Figure 8: Thin Layer Chromatography Setup (Created on Chemix)

Current-day uses of Chromatography

Chromatography is a versatile separation technique that has found wide-ranging applications in various fields of research, including pharmaceuticals, biochemistry, environmental science, food and beverage analysis, and forensic analysis. In this method, a stationary phase and a mobile phase are used to separate a mixture of components based on their physical and chemical properties. The mobile phase is a fluid that carries the sample through the stationary phase while the stationary phase is a substance that stays fixed in place. As the mobile phase moves through the stationary phase, each component in the mixture interacts differently with the stationary phase, leading to their separation. In this essay, we will discuss the current-day uses of chromatography in detail.

Pharmaceutical Industry

Chromatography has been extensively used in the analysis of pharmaceuticals and drugs. The technique is used to separate, identify, and quantify the active components in a drug formulation, impurities, and degradation products. High-performance liquid chromatography, or HPLC, is one of the chromatographic methods frequently used in pharmaceutical analysis. Drugs like analgesics, antibiotics, antivirals, antidepressants, and antihypertensives are just a few of the many types of medications that can be analyzed using HPLC (Chambers, 2009). HPLC is preferred in pharmaceutical analysis because it is sensitive, selective, and reproducible.

Separation of Biomolecules

Chromatography is an essential technique in the separation and purification of proteins and amino acids. Proteins are complex biomolecules composed of amino acid building blocks. The analysis of proteins requires their isolation and purification from the sample matrix. Proteins are separated from one another and purified using chromatography methods like ion exchange chromatography, size exclusion chromatography, and affinity chromatography(Maheshwari, 2013). These techniques are based on the differences in the physicochemical properties of proteins, such as charge, size, and affinity for ligands.

Pollutant detection

Chromatography has become an indispensable technique in the identification and quantification of pollutants in water and air. The technique is used to analyze a wide range of environmental contaminants, including pesticides, herbicides, polycyclic aromatic hydrocarbons (PAHs), and volatile organic compounds (VOCs) (Suryani et al., 2020). Gas chromatography (GC) and liquid chromatography (LC) are commonly used chromatographic techniques in environmental analysis. These techniques offer high sensitivity, selectivity, and accuracy, making them ideal for the analysis of trace-level environmental contaminants.

Testing Food and Beverage Contamination

In the food and beverage industry, chromatography is frequently used to analyze contaminants like pesticides, mycotoxins, and food additives. The technique is used to analyze food samples, such as fruits, vegetables, cereals, dairy products, and beverages. Chromatography techniques such as HPLC, GC, and thin-layer chromatography (TLC) are used in food and beverage analysis (Ghoul et al., 2020). These techniques offer high selectivity, sensitivity, and accuracy, making them ideal for the detection of trace-level contaminants.

Analyzing DNA and other biomolecules

Chromatography has found wide-ranging applications in the analysis of DNA and other biomolecules. DNA is a complex biomolecule composed of nucleotide building blocks. The analysis of DNA requires the separation and purification of the DNA from the sample matrix. For the separation and purification of DNA, chromatography methods like ion exchange chromatography, size exclusion chromatography, and affinity chromatography are employed. The differences in the physicochemical characteristics of DNA, such as charge, size, and affinity for ligands, are the foundation of these methods. Other biomolecules like metabolites, lipids, and carbohydrates are also analyzed using chromatography (Rivas et al., 2021).

Forensic Sciences

Chromatography is an essential tool in forensic analysis, especially when testing for drugs. Chromatography is used in drug testing to find and measure drug compounds in bodily fluids like blood, urine, and saliva. Drug testing frequently uses chromatography techniques like gas chromatography and high-performance liquid chromatography (HPLC).

In forensic analysis, chromatography is also used to identify and analyze chemical compounds found at crime scenes, such as accelerants used in arson or explosives. Chromatography can also help identify the source of drugs found at a crime scene, which can be used to trace drug trafficking networks (Caddy, 2020).

Table1: Summarized information related to the various Chromatographic Techniques discussed above

Chromatography Type	**Scientist(s) Discovered**	**Year of Discovery**	**Applications**	**References**
Paper Chromatography	Martin and Synge	1941	Separation and identification of components of a mixture	Pavia et al., 2010
Gas Chromatography	Archer John Porter Martin and Richard Laurence Millington Synge	1941	Separation and analysis of volatile and semi-volatile compounds	Skell, 2016
Liquid Chromatography	Tswett, Richard Kuhn, and Edgar Lederer	1906-1940s	Separation and analysis of organic and inorganic compounds	Majors, 2011
Ion Chromatography	Small, Stevens, and Bauman	1975	Separation and analysis of ions	El Rassi, 2017
High-performance Liquid Chromatography (HPLC)	Csaba Horvath	1967	Separation and analysis of complex mixtures	Majors, 2011
Size-exclusion Chromatography	Raymond D. Lundberg and Per Flodin	1955	Separation and purification of proteins, peptides, and other biomolecules	Volk, 2011
Affinity Chromatography	Pedro Cuatrecasas	1970	Isolation and purification of proteins and other biomolecules based on specific	Kohoutova & Brabcova, 2019

			interactions	
Chiral Chromatography	HPLC (Mid-1970s), GC (1990s)	Mid-1970s/1990s	Separation and analysis of enantiomers	Zhang et al., 2019

Future Aspects

With the development of new technologies, the future of chromatography looks promising. One of the most exciting developments is the integration of chromatography with other techniques such as mass spectrometry and NMR spectroscopy (Novotny & Samec, 2011). These advances allow for more precise identification and quantification of analytes in complex mixtures, making chromatography an even more powerful tool in fields such as proteomics, metabolomics, and drug discovery.

Another exciting development in chromatography is the advent of new materials with improved separation capabilities. These materials include monoliths, which are porous structures that offer high efficiency and resolution, and surface-modified particles, which can selectively capture and separate specific molecules from complex mixtures (Giddings, 2003). These new materials offer improved separation capabilities and increased sensitivity, making chromatography an even more valuable tool for research and analysis.

Finally, the miniaturization of chromatography systems is also a promising future aspect of the technique (Ramsey & Ramsey, 2003). Miniaturization allows for faster analysis times, lower solvent consumption, and lower detection limits. This makes chromatography more accessible to a wider range of applications and allows for on-site analysis in fields such as environmental monitoring and food safety.

Overall, the future of chromatography looks bright with the integration of new technologies, the development of new materials, and the miniaturization of systems. These advancements will continue to improve the precision, sensitivity, and efficiency of chromatography, making it an even more valuable tool for research and analysis in a variety of fields.

Conclusion

In summary, chromatography is a technique that has a long and rich history, beginning with its discovery in the early 1900s by Mikhail Tsvet. Over the years, various types of chromatography have been developed to cater to specific needs and applications, such as gas chromatography, liquid chromatography, and ion exchange chromatography.

Chromatography has become a crucial tool in modern scientific research and development due to its ability to separate and analyze complex mixtures of chemicals. It has uses in a number of different disciplines, including biochemistry, pharmaceuticals, forensics, food science, and environmental science, among others.

One of the key benefits of chromatography is its ability to separate and purify molecules with high accuracy and precision, which is essential for the development of new drugs and therapies.

It has also helped in the identification of new compounds and the elucidation of their structures, leading to a better understanding of biological processes.
In conclusion, chromatography has had a significant impact on modern science, and its importance is only expected to increase in the future as new and more complex analytical challenges arise.

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