GAS AND LIQUID CHROMATOGRAPHY

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Summary

Chromatography is the most important technique of separation of chemical substances, and is based on the physical interaction of a solute with two different phases. From its discovery it has been developed continuously in all branches, particularly in materials and the refinement of instrumentation, which has resulted in the efficient, reliable, and very sensitive chromatographic methods that are widespread in all modern analytical laboratories. In this article we will be describing the principal chromatographic techniques, with particular attention to instrumental gas and liquid chromatography.

1. Introduction

Few methods of chemical analysis are truly specific to a particular analyte. It is often found that the analyte of interest must be separated from the myriad of individual compounds that may be present in a sample. As well as providing the analytical scientist with methods of separation, chromatographic techniques can also provide methods of analysis.

Mikhail Semyonovich Tswett (1872–1919), a Russian botanist, is regarded as the father of chromatography as a result of experiments in which he used a chalk column to separate pigments in green leaves. Tswett first presented the basic principles of his separation method in 1906, then published two papers in 1906 in which he called the method chromatography. In his original experiments, Tswett tamped a fine powder, such as sucrose, into a glass tube to produce a column of the desired height. After extracting the pigments from leaves and transferring them to petroleum ether, he poured a small volume of the solution onto the column. When the solution had percolated and formed a narrow initial zone beneath the top of the adsorbent, fresh solvent (e.g., petroleum ether) was added and pressure applied to the top of the column. As the solvent flowed through the column the individual pigments moved at different rates and eventually separated from each other. The key features of Tswett's technique were the application of the mixture as a narrow initial zone and the development of the chromatogram by application of fresh solvent. Other early workers had employed procedures based on the phenomena of adsorption or partition, but these lacked Tswett's critical development step, and therefore did not yield extensive resolution of the mixtures. Since Tswett's separation of plant pigments was visually measured by varying colors, it is widely believed the scientist combined two Greek words (*chroma* meaning "color" and *graphos* meaning "written") to form the new word meaning "color writing." However, since the name Tswett means "color" in Russian, it may well be the botanist was simply naming the process after himself.

Chromatography involves a sample, or sample extract, being dissolved in a mobile phase, which may be a gas, a liquid, or a supercritical fluid. The mobile phase is then forced through an immobile, immiscible stationary phase. The phases are chosen such that components of the sample have differing solubilities in each phase. A component that is quite soluble in the stationary phase will take longer to travel through it than a component that is not very soluble in the stationary phase but very soluble in the mobile

phase. As a result of these differences in mobilities, sample components will become separated from each other as they travel through the stationary phase. The theoretical aspects of chromatography were first studied by Wilson in 1940, who discussed the quantitative aspects in terms of diffusion, rate of adsorption, and isotherm nonlinearity. The first comprehensive mathematical treatment describing column performance (using the height equivalent to a theoretical plate), in terms of stationary phase particle size and diffusion, was presented in 1949. However, it was J.J. van Deemter and co-workers in 1956 who developed the rate theory to describe the separation processes, following on from the earlier work—in 1952—of Leon Lapidus and Neal Amundson. John Calvin Giddings first looked at the dynamic theory of chromatography in 1955, while from the 1960s onward he has examined many aspects of gas chromatography and general chromatography theory. It was from this basis that modern chromatography has developed. There has been continuous development in all branches of chromatography, particularly in materials and the refinement of instrumentation, which has resulted in the efficient, reliable, and sensitive chromatographic methods in use today, and which form the backbone of modern analytical procedures and routine laboratory analysis.

2. Evolution and Classification of Chromatography

Tswett's original column-adsorption chromatographic method has been modified in many ways, resulting in the different types of chromatography. Liquid chromatography (LC) is an analytical chromatographic technique that is useful for separating ions or molecules that are dissolved in a solvent. If the sample solution is in contact with a second solid or liquid phase, the different solutes will interact with the other phase to differing degrees due to differences in adsorption, ion-exchange, partitioning, or size. These differences allow the mixture components to be separated from each other by using these differences to determine the transit time of the solutes through a column.

Simple LC consists of a column with a fritted (porous glass) bottom that holds a stationary phase in equilibrium with a solvent. Typical stationary phases—and their interactions with the solutes—are:

- solids (adsorption),
- ionic groups on a resin (ion exchange),
- liquids on an inert solid support (partitioning), and
- porous inert particles (size exclusion).

The mixture to be separated is loaded onto the top of the column followed by more solvent. The different components in the sample mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase. The compounds are separated by collecting aliquots of the column effluent as a function of time, as shown in Figure 1.

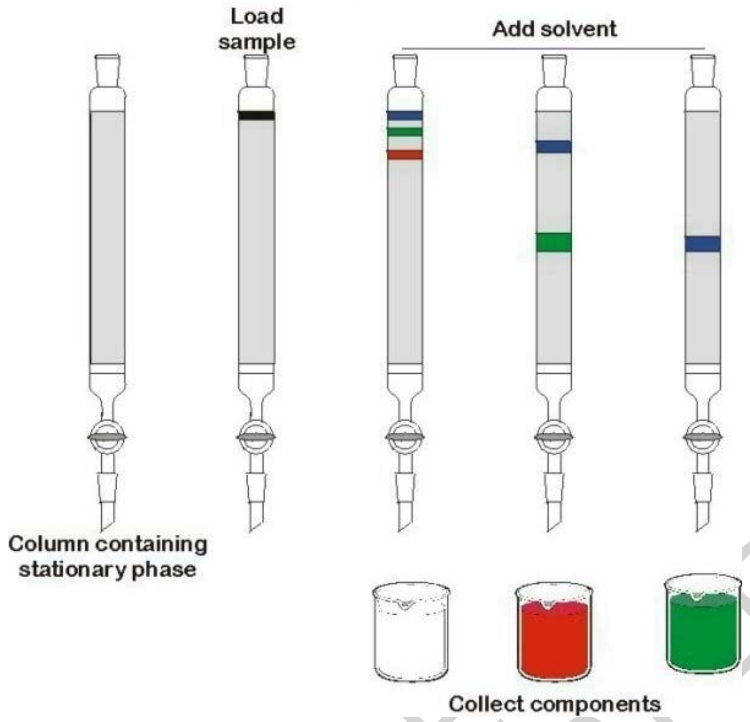

Figure 1. Classical separation obtained by liquid chromatography (LC) on column

Conventional LC is most commonly used in preparative scale work to purify and isolate some components of a mixture. It is also used in ultratrace separations where small disposable columns are used once and then discarded. Analytical separations of solutions for detection or quantification typically use more sophisticated high-performance liquid chromatography (HPLC) instruments. HPLC instruments use a pump to force the mobile phase through, and provide higher resolution and faster analysis time.

Thin-layer chromatography (TLC) is a chromatographic technique that is useful for separating organic compounds. Because of the simplicity and rapidity of TLC, it is often used to monitor the progress of organic reactions and to check the purity of products. TLC consists of a stationary phase immobilized on a glass or plastic plate, and an organic solvent. The sample, either liquid or dissolved in a volatile solvent, is deposited as a spot on the stationary phase. The constituents of a sample can be identified by simultaneously running standards with the unknown. The bottom edge of the plate is placed in a solvent reservoir, and the solvent moves up the plate by capillary action. When the solvent front reaches the other edge of the stationary phase, the plate is removed from the solvent reservoir. The separated spots are visualized with ultraviolet light or by placing the plate in iodine vapor. The different components in the mixture move up the plate at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase. TLC can be automated using forced solvent flow, running the plate in an vacuum-capable chamber to dry the plate, and recording the finished chromatogram by absorption or fluorescence spectroscopy with a light source. The ability to program the solvent delivery makes it convenient to do multiple developments in which the solvent flows for a short period of time, the TLC plate is dried, and the process is repeated. This method refocuses the spots to achieve

higher resolution than in a single run. Two-dimensional TLC uses the TLC method twice to separate spots that are unresolved by only one solvent. After running a sample in one solvent, the TLC plate is removed, dried, rotated 90°, and run in another solvent. Any of the spots from the first run that contain mixtures can now be separated. The finished chromatogram is a two-dimensional array of spots.

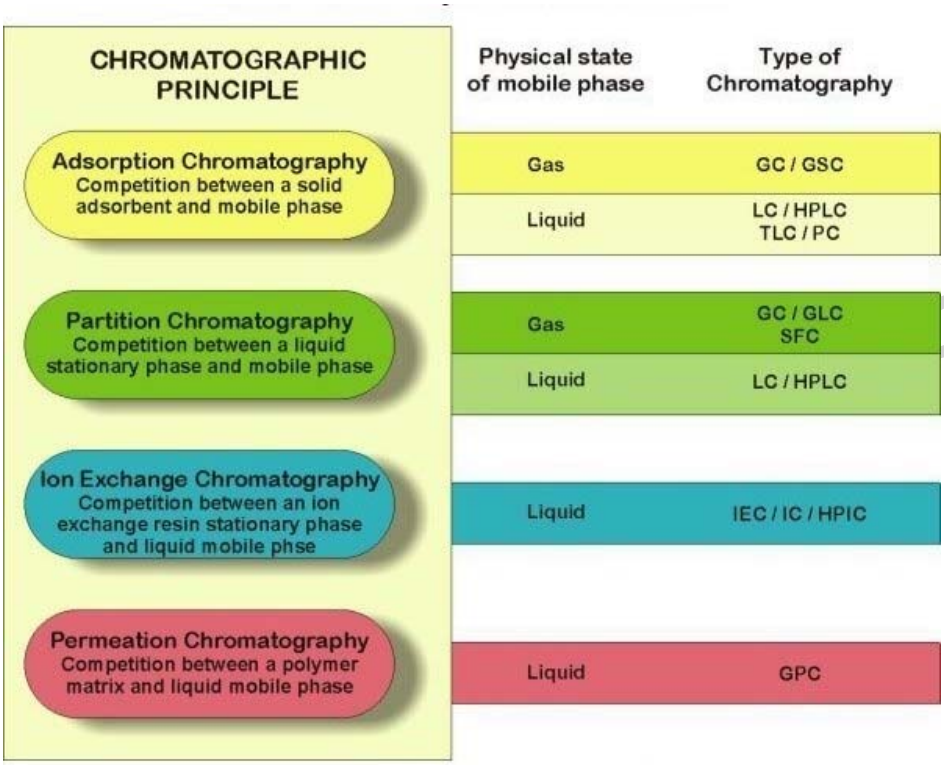

Figure 2. Classification of chromatographic techniques

In recent years, the main chromatographic techniques include paper chromatography (PC), TLC, ion-exchange chromatography (IEC), gel-permeation chromatography (GPC) (size exclusion), affinity chromatography, gas chromatography (GC), supercritical-fluid chromatography (SFC), HPLC, and capillary (zone) electrophoresis (CE). Techniques such as HPLC and GC use columns—narrow tubes packed with the stationary phase—through which the mobile phase is forced. The sample is transported through the column by continuous addition of the mobile phase. This process is called elution. The average rate at which an analyte moves through the column is determined by the time it spends in the mobile phase. The individual types of chromatographic techniques are shown in Figure 2.

As mentioned above, chromatographic processes can be classified according to the type of equilibration process involved, which is governed by the type of stationary phase. The various bases of equilibration are:

- adsorption
- partition
- ion exchange
- pore penetration

- affinity chromatography.

2.1. Adsorption Chromatography

The stationary phase is a solid on which the sample components are adsorbed. The mobile phase may be a liquid (liquid–solid chromatography) or a gas (gas–solid chromatography); the components distribute between the two phases through a combination of sorption and desorption processes. TLC is a special example of sorption chromatography in which the stationary phase is a plane, in the form of a solid supported on an inert plate.

2.2. Partition Chromatography

Partition chromatography depends on the partition, or distribution, of each component of a mixture between two immiscible phases. One of the liquids is held stationary by strong adsorption on the surface of a finely divided solid or on the inside wall of a capillary column, while the other flows through it. Any substance that preferentially dissolves in the mobile phase is more rapidly transported in the direction of flow than is a substance that has greater affinity for the stationary liquid. Again, the mobile phase may be a liquid (liquid–liquid partition chromatography) or a gas (gas–liquid chromatography (GLC)). PC is a type of partition chromatography in which the stationary phase is a layer of water adsorbed on a sheet of paper. In the normal mode of operations of liquid–liquid partition, a polar stationary phase (e.g., water or methanol) is used with a nonpolar stationary phase (e.g., hexane). This favors retention of polar compounds and elusion of nonpolar compounds, and is called normal-phase chromatography. If a nonpolar stationary phase is used with a polar mobile phase, then nonpolar solutes are retained more and polar solutes more readily eluted. This is called reversed-phase chromatography.

2.3. Ion-Exchange and Size-Exclusion Chromatography

IEC uses an ion-exchange resin as the stationary phase. The mechanism of separation is based on ion-exchange equilibria. When a chromatographic format is chosen for the analysis of an ionic compound, ion exchange is generally considered after attempts at developing a reversed-phase or reversed-phase, ion-pair method have proved unsuccessful. However, IEC is the method of choice for the analysis of inorganic ions, and it is often preferable to reversed-phase methods for the analysis of small organic ions. In size-exclusion chromatography, solvated molecules are separated according to their size by their ability to penetrate a sieve-like structure—the stationary phase. Separation in partition chromatography and IEC arises from different interactions of the solutes with the mobile phase and the stationary phase. In contrast, separations in size-exclusion chromatography arise from differences in molecular size and the ability of different molecules to penetrate the pores of the stationary phase to different extents. Size-exclusion chromatography is used extensively for the preparative separations of macromolecules of biological origin, as well as for the purification of synthetic-organic polymers.

2.4. Affinity Chromatography

This newest and most selective kind of chromatography utilizes highly specific interactions between one kind of solute molecule and a second molecule covalently attached (immobilized) to the stationary phase. For example, the immobilized molecule might be an antibody to a particular protein. When a crude mixture containing 1000 proteins is passed through the column, only the one protein that reacts with the antibody is bound to the column. After washing all the other solutes off the column, the desired protein is dislodged from the antibody by changing the pH or ionic strength. These are arbitrary classifications of chromatographic techniques, and some types of chromatography are considered together as a separate technique, such as GC for gas–solid and gas–liquid chromatography. In every case, successive equilibria are at work that determine to what extent the analyte stays behind or moves along with the eluent—the mobile phase. The mobile phase can be a liquid or a gas, and the stationary phase a liquid or a solid. Separation involving two immiscible liquid phases is referred to as partition or liquid–liquid chromatography (LLC), that is, a solute is partitioned between a liquid-mobile phase and liquid-film stationary phase. When physical surface forces control the retention properties of the component on a solid stationary phase, liquid–solid (adsorption) chromatography (LSC) occurs; correspondingly, when the mobile phase is a gas we have GLC and gas–solid chromatography (GSC), respectively.

3. Chromatographic Theory

3.1. Distribution of Analytes Between Phases

The distribution of analytes between phases can often be described quite simply. An analyte is in equilibrium between the two phases, as illustrated in Figure 3.

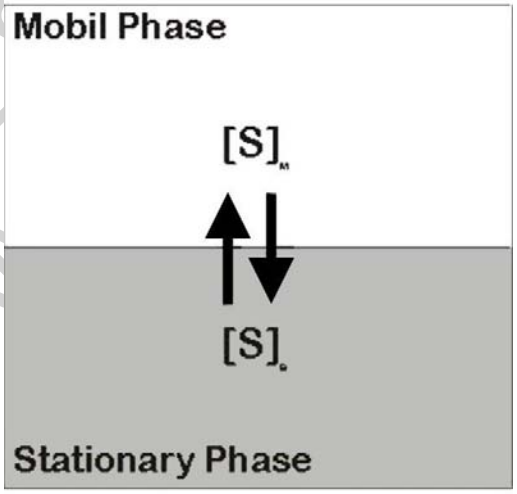

Figure 3. Distribution of analytes between two phases

The equilibrium constant, *K*, is termed the partition coefficient; defined as the molar concentration of the analyte in the stationary phase divided by the molar concentration of the analyte in the mobile phase. Each component separated will have a different value for *K*, reflecting their relative affinities for the stationary phase. The generalized form of the distribution equation for each component is:

$$K = \frac{C_S}{C_M} \tag{1}$$

where C_S is the concentration of a component in the stationary phase/unit volume, and C_M is the concentration of a component in the mobile phase/unit volume. The time between sample injection and an analyte peak reaching a detector at the end of the column is termed the retention time (t_R). Each analyte in a sample will have a different retention time. The time taken for the mobile phase to pass through the column is called t_M.

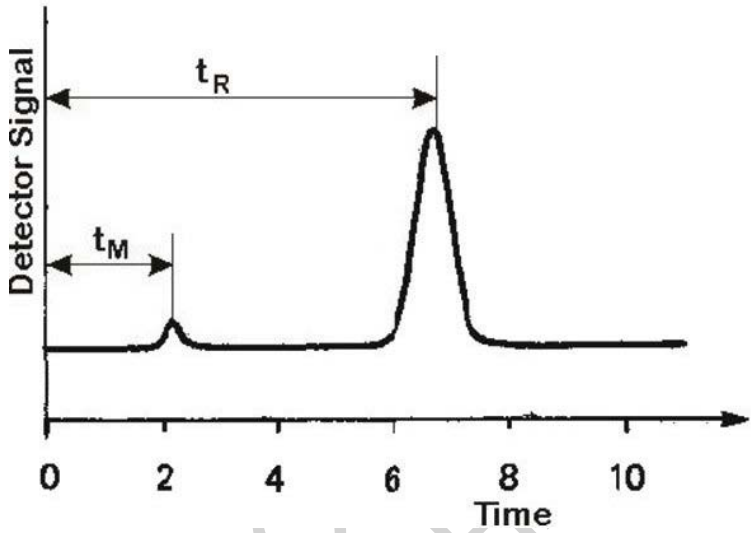

Figure 4. Typical chromatogram

A term called the retention factor, *k'*, is often used to describe the migration rate of an analyte on a column. You may also find it called the capacity factor. The retention factor for analyte, *A*, is defined as:

$$k'_A = (t_R - t_M)/t_M \tag{2}$$

t_R and t_M are easily obtained from a chromatogram. The difference $(t_R - t_M)$ is called the adjusted retention time. When an analyte's retention factor is less than 1, elution is so fast that accurate determination of the retention time is very difficult. High retention factors—greater than 20—mean that elution takes a very long time. Ideally, the retention factor for an analyte is between 1 and 5. A quantity called the selectivity factor, α, which describes the separation of two species (*A* and *B*) on the column is define as:

$$\alpha = \frac{k'_B}{k'_A} \tag{3}$$

When calculating the selectivity factor, species *A* elutes faster than species *B*. The selectivity factor is always greater than 1. Chromatography encompasses a number of variations on the basic principles of the separation of components in a mixture achieved by a successive series of equilibrium stages. These equilibria depend on the partition or

differential distribution of the individual components between two phases: a mobile phase that moves over a stationary phase.

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Biographical Sketches

Davino Gelosa was born in Monza (Milano), Italy, on December 12 1948, and graduated in Agrarian and Natural Science at the University of Milano in 1978. He was technical official at the Politecnico of Milano from 1968 to 1986, then technical coordinator at the Department of Chimica Fisica Applicata (Politecnico of Milano), where he is still working in charge of Chemistry and Physics–Chemistry Laboratories. His research was initially in the field of catalysis, then his research interest focused on some aspects of applied kinetics in chemical and adsorption reactions. His working activity has been largely connected with the employment of the main laboratory techniques and instruments. He has contributed about 40 papers to international journals and four patents. He has been a consultant for different companies on the development of industrial processes. He is married, with one son. His preferred sports are running and cross-country skiing.

Andrea Sliepcevich was born in Milano, Italy, on November 22 1968, and graduated in Industrial Chemistry at the University of Milano in 1995. He obtained his Ph.D. in Electrochemical Engineering at

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