Chromatography

Chromatography, technique for separating the components, or solutes, of a mixture on the basis of the relative amounts of each solute distributed between a moving fluid stream, called the mobile phase, and a contiguous stationary phase. The mobile phase may be either a liquid or a gas, while the stationary phase is either a solid or a liquid.

Kinetic molecular motion continuously exchanges solute molecules between the two phases. If, for a particular solute, the distribution favours the moving fluid, the molecules will spend most of their time migrating with the stream and will be transported away from other species whose molecules are retained longer by the stationary phase. For a given species, the ratio of the times spent in the moving and stationary regions is equal to the ratio of its concentrations in these regions, known as the partition coefficient. (The term *adsorption isotherm* is often used when a solid phase is involved.) A mixture of solutes is introduced into the system in a confined region or narrow zone (the origin), whereupon the different species are transported at different rates in the direction of fluid flow. The driving force for solute migration is the moving fluid, and the resistive force is the solute affinity for the stationary phase; the combination of these forces, as manipulated by the analyst, produces the separation.

Chromatography is one of several separation techniques defined as differential migration from a narrow initial zone. Electrophoresis is another member of this group. In this case, the driving force is an electric field, which exerts different forces on solutes of different ionic charge. The resistive force is the viscosity of the nonflowing solvent. The combination of these forces yields ion mobilities peculiar to each solute.

As a separation method, chromatography has a number of advantages over older techniques—crystallization, solvent extraction, and distillation, for example. It is capable of separating all the components of a multicomponent chemical mixture without requiring an extensive foreknowledge of the identity, number, or relative amounts of the substances present. It is versatile in that it can deal with molecular species ranging in size from viruses composed of millions of atoms to the smallest of all molecules—hydrogen—which contains only two; furthermore, it can be used with large or small amounts of material. Some forms of chromatography can detect substances present at the attogram (10^{-18} gram) level, thus making the method a superb trace analytical technique extensively used in the detection of chlorinated pesticides in biological materials and the environment, in forensic science, and in the detection of both therapeutic and abused drugs. Its resolving power is unequaled among separation methods.

History

Early developments

The first purely pragmatic application of chromatography was that of the early dye chemists, who tested their dye mixtures by dipping strings or pieces of cloth or filter paper into a dye vat. The dye solution migrated up the inserted material by capillary action, and the dye components produced bands of different colour. In the 19th century, several German chemists carried out deliberate experiments to explore the phenomenon. They observed, for example, the development of concentric coloured rings by dropping solutions of inorganic compounds onto the centre of a piece of filter paper; a treatise was

published in 1861 by Friedrich Goppelsröder describing the method and giving it the name "capillary analysis."

The discovery of chromatography, however, is generally attributed to the Russian botanist Mikhail S. Tsvet (also spelled Tswett), because in 1901 he recognized the physicochemical basis of the separation and applied it in a rational and organized way to the separation of plant pigments, particularly the carotenoids and the chlorophylls. Tsvet described a technique that is used today in essentially the same form. He packed a vertical glass column with an adsorptive material, such as alumina, silica, or powdered sugar, added a solution of the plant pigments to the top of the column, and washed the pigments through the column with an organic solvent. The pigments separated into a series of discrete coloured bands on the column, divided by regions entirely free of pigments. Because Tsvet worked with coloured substances, he called the method chromatography (from Greek words meaning colour writing). Tsvet's development of chromatographic procedures was generally unknown to chemists in the Western world because he published either in German botanical journals or in Russian works. In 1931 chromatography emerged from its relative obscurity when the German chemist Richard Kuhn and his student, the French chemist Edgar Lederer, reported the use of this method in the resolution of a number of biologically important materials. In 1941 two British chemists, Archer J.P. Martin and Richard L.M. Synge, began a study of the amino acid composition of wool. Their initial efforts, in which they used a technique called liquid-liquid countercurrent distribution, failed to give them adequate separation; they conceived, therefore, of an alternative method, in which one liquid was firmly bound to a finely granulated solid packed in a glass tube and a second liquid, immiscible with the first, was percolated through it. Silica gel served as the granular solid, and Martin and Synge pictured the gel as composed of water tightly bonded to the crystals of silica; the mobile phase was chloroform. Their work with this technique was remarkably successful. Although their method was mechanically identical with Tsvet's approach, it was innovative in that it involved the concept of a stationary liquid (water) supported on an inert solid (silica), with the result that the solute molecules partitioned between the stationary liquid and a separate mobile liquid phase (chloroform). The technique came to be called partition chromatography. At that time, Martin and Synge suggested that the moving phase could well be a gas. It is a historical oddity that this idea was overlooked for nearly a decade, possibly because of the war, until Martin in collaboration with the British chemist Anthony T. James initiated studies of gas-liquid partition chromatography. In 1952 Martin and Synge were awarded the Nobel Prize for their work, perhaps not so much for the newness of the technique but for a model that suggested other systems, a mathematical theory, and an applicability to amino acid and peptide separations with far-reaching impact on biochemical studies.

The initial partition-chromatography system presented difficulties because of lack of reproducibility in the properties of the silica gel and lack of uniformity in the packing of columns. Partly for this reason, Martin and his coworkers worked out a new procedure in which the stationary medium was a sheet of filter paper. The paper was thought of as water bonded to cellulose, providing another partition method. The technique gave the desired reproducibility, and beginning in the 1940s paper chromatography found wide application in the analysis of biologically important compounds, such as amino acids, steroids, carbohydrates, and bile pigments. In this field it replaced, to a large extent, the column technique initiated by Tsvet.

Motivated probably by the same drawbacks to column chromatography, two Soviet pharmacists, Nikolay A. Izmaylov and Maria S. Shrayber, distributed the support material as a thin film on a glass plate. The plate and support material could then be manipulated in a fashion similar to that of paper chromatography. The results of the Soviet studies were reported in 1938, but the potential of the method was not widely realized until 1956, when the German chemist Egon Stahl began intensive research on its application. This system became known as thin-layer chromatography (TLC).

Still another chromatographic technique, gas chromatography, was first carried out in Austria in 1944 by the chemist Erika Cremer, who used a solid stationary phase. The first extensive exploitation of the method was made by Martin and James in 1952, when they reported the elution gas chromatography of organic acids and amines. In this work, small particles of support material were coated with a nonvolatile liquid and packed into a heated glass tube. Mixtures injected into the inlet of the tube and driven through by compressed gas appeared in well-separated zones. This development was immediately recognized by petroleum chemists as a simple and rapid method of analysis of the complex hydrocarbon mixtures encountered in petroleum products. British Petroleum and Shell Oil Company laboratories immediately began basic research in their own laboratories. Instrument companies, sensing an extensive market, also made major contributions.

Subsequent developments

In 1957, while doing a theoretical study of gas chromatographic columns, Marcel J.E. Golay, as a consultant for the Perkin-Elmer Corporation, concluded that a very long column (90 to 180 metres [300 to 600 feet]) of narrow-diameter tubing (internal diameter of 0.25 millimetres [0.0098 inch]) with its wall coated with a thin film of liquid would yield superior separations. Fortunately, at about this same time, detectors with extremely low limits of detection became available, which could sense the small sample sizes required by these new columns. These capillary, or Golay, columns, now called open-tubular columns and characterized by their open design and an internal diameter of less than one millimetre, had an explosive impact on chromatographic methodology. It is now possible to separate hundreds of components of a mixture in a single chromatographic experiment.

Molecular sieves are porous substances that trap a mobile-phase gas. Large molecules cannot enter the pores, and so they flow largely unimpeded through the system. Small molecules are interrupted in their migration as they meander in and out of the pores by diffusion. Molecules of intermediate sizes show different rates of migration, depending on their size. In 1959 Per Flodin and Jerker Porath in Sweden developed cellulose polymeric materials that acted as molecular sieves for substances dispersed in liquids. This extended the molecular weight range of chromatography to polypeptides, proteins, and high-molecular-weight polymers. The generic term for such separations is size-exclusion chromatography.

In 1964 the American chemist J. Calvin Giddings, referring to a theory largely worked out for gas chromatography, summarized the necessary conditions that would give liquid chromatography the resolving power achievable in gas chromatography—that is, very small particles with a thin film of stationary phase in small-diameter columns. The development of the technique now termed high-performance liquid chromatography (HPLC) depended on (1) the development of pumps that would deliver a steady stream of liquid at high pressure to the column to force the liquid through the narrow interstitial channels of the packed columns at reasonable rates, and (2) detectors that would sense the small sample sizes mandated. At first, only adsorptive solids were used as the stationary phase, because

liquid coatings were swept away by the mobile phase. Previously gas chromatography had employed chemical bonding of an organic stationary phase to solids to reduce adsorptive activity; István Halász of Germany exploited these reactions to cause a separation based on liquid solution effects in the bonded molecular layers. These and similar reactions were employed to give firmly attached molecules that acted as a thin film of solvent in liquid systems. These bonded phases gave high-performance liquid chromatography such scope and versatility that the technique is now the dominant method for separations.

Ion-exchangers are natural substances—for example, certain clays—or deliberately synthesized resins containing positive ions (cation exchangers) or negative ions (anion exchangers) that exchange with those ions in solution having a greater affinity for the exchanger. This selective affinity of the solid is called ion, or ion-exchange, chromatography. The first such chromatographic separations were reported in 1938 by T.I. Taylor and Harold C. Urey, who used a zeolite. The method received much attention in 1942 during the Manhattan Project as a means of separating the rare earths and transuranium elements, fission products of uranium, and other elements produced by thermonuclear explosions. Ion-exchange chromatography can be applied to organic ion separations and has particular importance for the separation of amino and nucleic acids.

As early as 1879, the solubility of solids in gases at high pressure had been observed. In 1958 the British scientist James Lovelock suggested that gases above their critical temperature (i.e., the temperature above which the appearance of a liquid phase cannot be produced by increasing the pressure) might be used at high pressure as mobile phases. A substance in this state is termed a supercritical fluid. At very high pressure, the density of the fluid can be 90 percent or more of the liquid density. The German chemist Ernst Klesper and his colleagues working at Johns Hopkins University were the first to report separation of the porphyrins with dense gases in 1962. Carbon dioxide at 400 atmospheres is a typical supercritical-fluid mobile phase. (One atmosphere equals 760 millimetres, or 29.92 inches, of mercury; standard sea-level pressure is one atmosphere.) In an extreme case, Giddings and his group used gases at pressures of up to 2,000 atmospheres to chromatograph carotenoids, sugars, nucleosides, amino acids, and polymers. Supercritical-fluid chromatography bridges a gap between gas chromatography and liquid chromatography. In gas chromatography, concentration of solutes in the gas phase is achieved with increased temperature. Supercritical-fluid chromatography achieves this result with increased pressure so that thermally unstable compounds may be analyzed. Additional advantages include increased speed and resolution.

A technique exhibiting great selectivity, affinity chromatography, was first described by Pedro Cuatrecasas and his coworkers in 1968. In these separations, a biomolecule such as an enzyme binds to a substrate attached to the solid phase while other components are eluted. The retained molecule can subsequently be eluted by changing the chemical conditions of the separation.

Another separation technique is based on the fact that the velocity of a fluid through a tube is not uniform. In the region immediately adjacent to the wall the fluid is nearly stationary. At distances farther from the wall, the velocity increases, reaching a maximum value at the centre of the channel. In 1966 Giddings conceived the idea that a field, electrical or gravitational, might be used to selectively attract particles to the wall, where they will move slowly through the system. Diffusion away from the high concentrations at the wall into faster inner streams would enhance migration. The net effect would yield differential migration. A thermal gradient between two walls has also been used. This technique is called field-flow

fractionation. It has been termed one-phase chromatography because there is no stationary phase. Its main applications are to polymers and particulate matter. The method has been used to separate biological cells, subcellular particles, viruses, liposomes, protein aggregates, fly ash, colloids, and pigments.

The battery of chromatographic techniques, along with field-flow fractionation, provides separations from the level of hydrogen molecules to particulates, encompassing a 10^{15}-fold mass range. An analogous mass range is one of grains of sand to boulders.

Principles of chromatography

Let's first familiarize ourselves with some terms that are commonly used in the context of chromatography:

Term	**Definition**
Mobile phase or carrier	solvent moving through the column
Stationary phase or adsorbent	substance that stays fixed inside the column
Eluent	fluid entering the column
Eluate	fluid exiting the column (that is collected in flasks)
Elution	the process of washing out a compound through a column using a suitable solvent
Analyte	mixture whose individual components have to be separated and analyzed

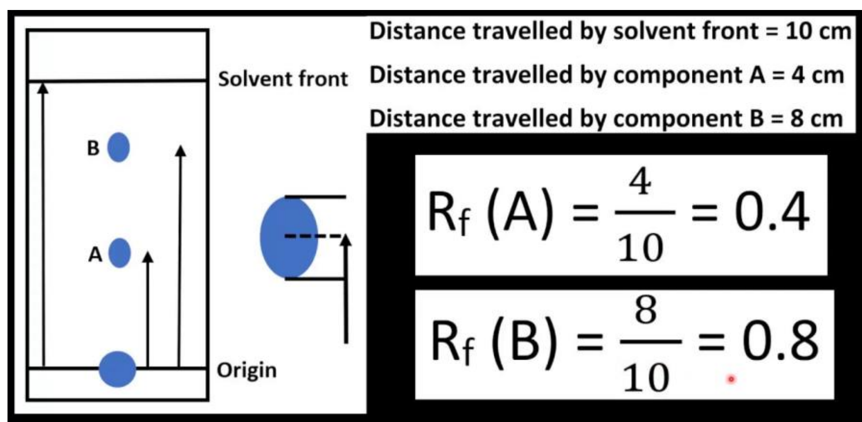
In all chromatography there is a mobile phase and a stationary phase. The stationary phase is the phase that doesn't move and the mobile phase is the phase that does move. The mobile phase moves through the stationary phase picking up the compounds to be tested. As the mobile phase continues to travel through the stationary phase it takes the compounds with it. At different points in the stationary phase the different components of the compound are going to be absorbed and are going to stop moving with the mobile phase. This is how the results of any chromatography are gotten, from the point at which the different components of the compound stop moving and separate from the other components. In paper and thin-layer chromatography the mobile phase is the solvent. The stationary phase in paper chromatography is the strip or piece of paper that is placed in the solvent. In thin-layer chromatography the stationary phase is the thin-layer cell. Both these kinds of chromatography use capillary action to move the solvent through the stationary phase.

What is the Retention Factor, R_f ?

The retention factor, R_f, is a quantitative indication of how far a particular compound travels in a particular solvent. The R_f value is a good indicator of whether an unknown compound and a known compound are similar, if not identical. If the R_f value for the unknown compound is close or the same as the R_f value for the known compound then the two compounds are most likely similar or identical. The retention factor, R_f, is defined as

R_f = distance the solute (D_1) moves divided by the distance traveled by the solvent front (D_2)

$R_f = D_1 / D_2$ where D_1 = distance that color traveled, measured from center of the band of color to the point where the food color was applied D_2 = total distance that solvent traveled



The Different Types of Chromatography

There are four main types of chromatography. These are Liquid Chromatography, Gas Chromatography, Thin-Layer Chromatography and Paper Chromatography.

Liquid Chromatography is used in the world to test water samples to look for pollution in lakes and rivers. It is used to analyze metal ions and organic compounds in solutions. Liquid chromatography uses liquids which may incorporate hydrophilic, insoluble molecules.

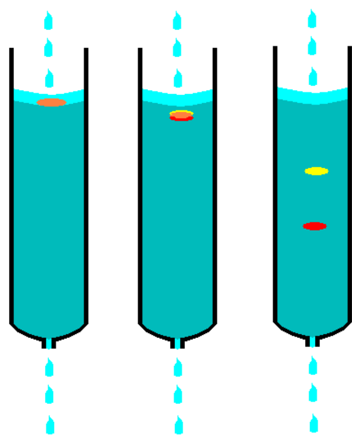
Gas Chromatography is used in airports to detect bombs and is used is forensics in many different ways. It is used to analyze fibers on a persons body and also analyze blood found at a crime scene. In gas chromatography helium is used to move a gaseous mixture through a column of absorbent material.

Thin-layer Chromatography uses an absorbent material on flat glass or plastic plates. This is a simple and rapid method to check the purity of an organic compound. It is used to detect pesticide or insecticide residuesin food. Thin-layer chromatography is also used in forensics to analyze the dye composition of fibers.

Paper Chromatography is one of the most common types of chromatography. It uses a strip of paper as the stationary phase. Capillary action is used to pull the solvents up through the paper and separate the solutes.

Column chromatography

Column chromatography is a chromatography technique used to separate mixture of chemical substances into its individual compounds. Column chromatography is a widely used method for the purification or separation of chemical compound mixture in lab.



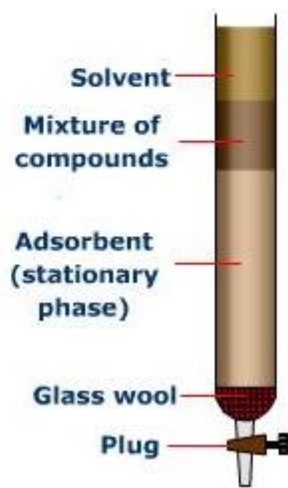
Principles of column chromatography

Column Chromatography consists of two phases: one mobile phase and one contiguous stationery phase. The stationery phase is solid and the mobile phase is liquid. The compound mixture moves along with the mobile phase through stationery phase and separates depending on the different degree of adhesion (to the silica) of each component in the sample or the compound mixture.

Explanation

The stationery phase

A glass tube with a circle large inlet and a small outlet with a plug or tap, named as column is used for this column chromatography. The column is placed vertically with a stand where the outlet is downward.



A piece of cotton wool is entered into the outlet and placed over the plug if there are no glass wool present to stop escaping the stationery phase from the column. There are two procedure to prepare the column by packing with silica or alumina:

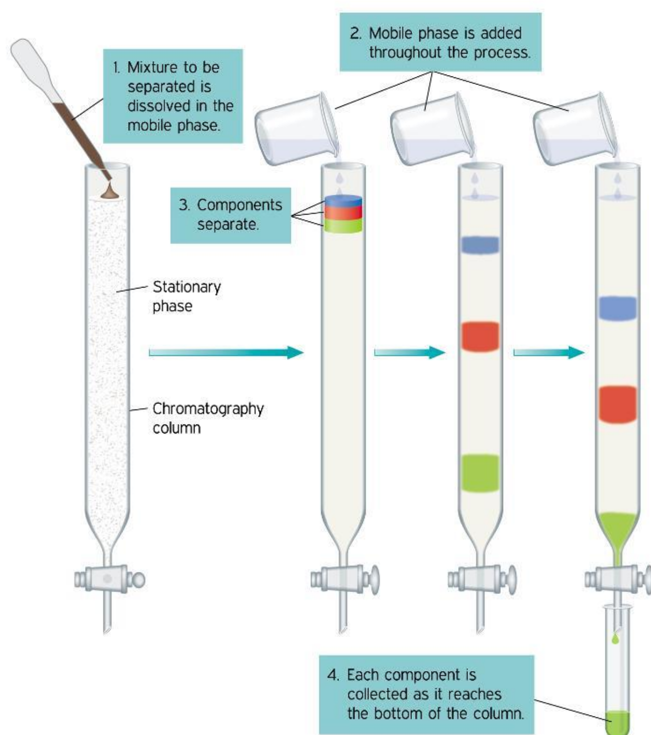
Dry method: In dry method at first the column is filled with dry powdered silica. Then the mobile phase, a suitable solvent is flushed through it until all the silica are wet and settled. From this point till the end always the column need to keep wet with solvent.

Wet method: In wet method firstly a slurry of silica and solvent is prepared and then poured onto the column using a funnel. More solvent must be used until the silica is settled into it.

Process

Column chromatography works in few steps:

Step 1: The mobile phase or eluent is either solvent or solvent mixture. The upper level of mobile phase should be same as the stationery phase. That means the stationery phase should be wet with the solvent. On this stage the compound mixture what need to be separated, are added from the top of the column in such a way that the top level of it is not disturbed. By turning on the tap below it is allowed to adsorbed on the surface of the silica.



Step 2: Then the solvent or a suitable solvent mixture is added at first touching the side of the glass column slowly and carefully so that the top level of the stationery phase is not disturbed. The solvent is repeatedly added as many times as needed throughout the process.

Step 3: When the tap, is on the compounds in the compound mixture move along with the eluent depending on the polarity of the sample molecule. The non polar components travel faster than the polar component.

Suppose if any compound mixture contains three compounds blue, red and green. According to polarity the order of these compounds are blue>red>green. That means blue is the most polar compound and thus will have less tendency to move along with the mobile phase.

Step 4: The green colored compound will travel first as it is less polar that other two. When it is near end of the column a clean test tube is taken to collect the green sample. After this the red and at last the most polar blue compound is collected, all in separate test tubes.

Thus a compound mixture is separated or purified by using column chromatography.

Summary

- Column layer chromatography is an chromatography technique used to separate mixture of chemical substances into its individual compounds.
- Chromatography consists of two phases: one mobile phase and one contiguous stationery phase.
- Column is prepared my mixing the silica with suitable solvent and poured in into a glass column.
- A suitable solvent (mobile phase) is moved along with compound mixture through the column according to the polarity.

Column Chromatography Applications

- Column Chromatography is used to isolate active ingredients.
- It is very helpful in Separating compound mixtures.
- It is used to determine drug estimation from drug formulations
- It is used to remove impurities.
- Used to isolation metabolites from biological fluids.

Types of Column Chromatography:

1. Adsorption column chromatography – Adsorption chromatography is a technique of separation, in which the components of the mixture are adsorbed on the surface of the adsorbent.

2. Partition column chromatography – The stationary phase, as well as mobile phase, are liquid in partition chromatography.

3. Gel column chromatography – In this method of chromatography, the separation takes place through a column packed with gel. The stationary phase is a solvent held in the gap of a solvent.

4. Ion exchange column chromatography – A chromatography technique in which the stationary phase is always ion exchange resin.

Thin Layer Chromatography

Thin Layer Chromatography is a technique used to isolate non-volatile mixtures. The experiment is conducted on a sheet of aluminium foil, plastic, or glass which is coated with a thin layer of adsorbent material. The material usually used is aluminium oxide, cellulose, or silica gel.

On completion of the separation, each component appears as spots separated vertically. Each spot has a retention factor (R_f) expressed as:

R_f = dist. travelled by sample / dist. travelled by solvent

The factors affecting retardation factor are the solvent system, amount of material spotted, absorbent and temperature. TLC is one of the fastest, least expensive, simplest and easiest chromatography technique.

Thin Layer Chromatography Principle

Like other chromatographic techniques, thin layer chromatography (TLC) depends on the separation principle. The separation relies on the relative affinity of compounds towards both the phases. The compounds in the mobile phase move over the surface of the stationary phase. The movement occurs in such a way that the compounds which have a higher affinity to the stationary phase move slowly while the other compounds travel fast. Therefore, the separation of the mixture is attained. On completion of the separation process, the individual components from the mixture appear as spots at respective levels on the plates. Their character and nature are identified by suitable detection techniques.

Thin Layer Chromatography Diagram

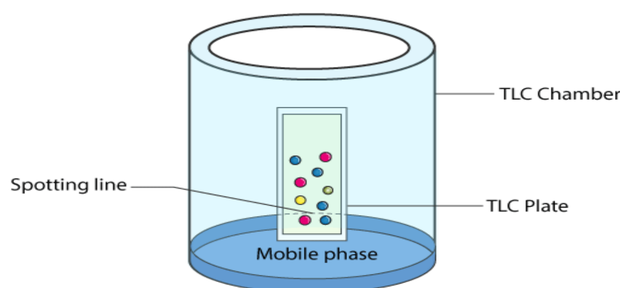


Diagram of Thin Layer Chromatography

Thin Layer Chromatography Procedure

Before starting with the Thin Layer Chromatography Experiment let us understand the different components required to conduct the procedure along with the phases involved.

1. Thin Layer Chromatography Plates – ready-made plates are used which are chemically inert and stable. The stationary phase is applied on its surface in the form of a thin layer. The stationary phase on the plate has a fine particle size and also has a uniform thickness.

2. Thin Layer Chromatography Chamber – Chamber is used to develop plates. It is responsible to keep a steady environment inside which will help in developing spots. Also, it prevents the solvent evaporation and keeps the entire process dust-free.

3. Thin Layer Chromatography Mobile phase – Mobile phase is the one that moves and consists of a solvent mixture or a solvent. This phase should be particulate-free. The higher the quality of purity the development of spots is better.

4. Thin Layer Chromatography Filter Paper – It has to be placed inside the chamber. It is moistened in the mobile phase.

Thin Layer Chromatography Experiment

The stationary phase that is applied to the plate is made to dry and stabilize.

- To apply sample spots, thin marks are made at the bottom of the plate with the help of a pencil.

- Apply sample solutions to the marked spots.

- Pour the mobile phase into the TLC chamber and to maintain equal humidity, place a moistened filter paper in the mobile phase.

- Place the plate in the TLC chamber and close it with a lid. It is kept in such a way that the sample faces the mobile phase.

- Immerse the plate for development. Remember to keep the sample spots well above the level of the mobile phase. Do not immerse it in the solvent.

- Wait till the development of spots. Once the spots are developed, take out the plates and dry them. The sample spots can be observed under a UV light chamber.

Thin Layer Chromatography Applications

- The qualitative testing of Various medicines such as sedatives, local anaesthetics, anticonvulsant tranquilisers, analgesics, antihistamines, steroids, hypnotics is done by TLC.

- TLC is extremely useful in Biochemical analysis such as separation or isolation of biochemical metabolites from its blood plasma, urine, body fluids, serum, etc.

- Thin layer chromatography can be used to identify natural products like essential oils or volatile oil, fixed oil, glycosides, waxes, alkaloids, etc

- It is widely used in separating multicomponent pharmaceutical formulations.

- It is used to purify of any sample and direct comparison is done between the sample and the authentic sample
- It is used in the food industry, to separate and identify colours, sweetening agent, and preservatives
- It is used in the cosmetic industry.
- It is used to study if a reaction is complete.

Disadvantages Of Thin Layer Chromatography:

1. Thin Layer Chromatography plates do not have longer stationary phase.
2. When compared to other chromatographic techniques the length of separation is limited.
3. The results generated from TLC are difficult to reproduce.
4. Since TLC operates as an open system, some factors such as humidity and temperature can be consequences to the final outcome of the chromatogram.
5. The detection limit is high and therefore if you want a lower detection limit, you cannot use TLC.
6. It is only a qualitative analysis technique and not quantitative.

Principles of paper chromatography

All chromatography follow the same principle. Paper Chromatography consists of two phases: one mobile phase and one contiguous stationery phase. The stationery phase a paper and the mobile gas is solvent. The compound mixture moves along with the mobile phase through stationery phase and separates depending on the different degree of adhesion (on the paper) of each component in the sample or the compound mixture.

Explanation

The stationery phase

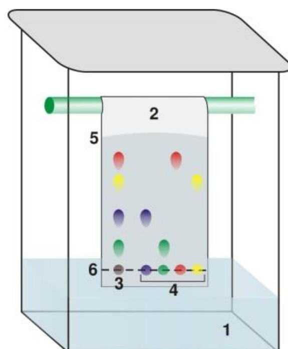
The paper chromatography is very similar to Thin layer chromatography. Difference is, instead of using a thin layer of silica on metal, it uses a special type of chromatography paper as stationery phase. This paper is made of cellulose. Cellulose is a polymer of simple sugar, glucose.

Cellulose contains -OH group similar to the silica or alumina on the TLC plate. The surface of cellulose is thus very polar. So the compounds can form hydrogen bond or can interact by van der waals dispersion forces and dipole dipole forces.

Process

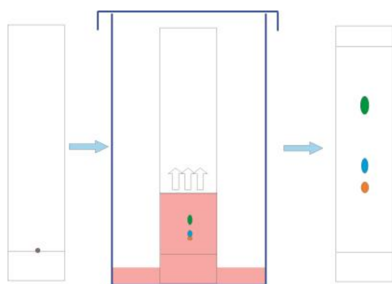
Paper chromatography works in few steps:

Step 1: A horizontal line is drawn near one end (about 1.5 cm from the bottom edge) of the paper. In figure below 6 is the horizontal line.



Step 2: The sample needs to be separated is placed as a small drop or line on to the paper using capillary tube. Labelling the drop by a pencil with an alphabet or number help to identify the compound later. In figure above 3 and 4 are the drops labelled. The drops are then soaked on the paper and dried.

Step 3: The paper is then placed into a sealed container with a swallow layer of suitable solvent. The solvent level must be lower than the pencil line or drop on it. The container need to be covered to stop the solvent to evaporate.

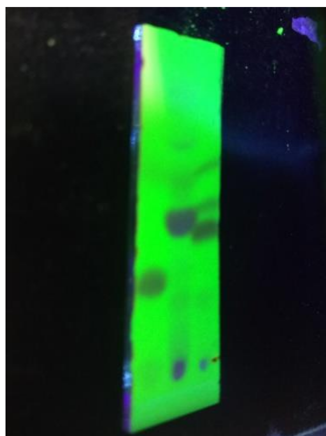


Step 4: The solvent rises up the paper chromatography taking each component of the sample with it. The components travel with the solvent depends on three things:

- The polarity of the sample molecule. The non polar components travel faster than the polar component.
- The attraction between the sample molecule and the solvent or solvent mixture.
- The attraction between the sample and the silica.

Suppose any sample compound mixture contains three colored molecules green, blue and red. According to their polarity, the order of these compounds is green<blue<red. Thus the most non polar green will travel first along with the mobile phase. Then blue and at last most polar compound the red one.

Step 5: When the solvent rises near the end of the paper then the paper should be taken out from sealed container and air dried. The paper with separated bands of components are then observed under UV-light.



R_f value

The compounds in the sample travels along with solvent to give separate bands on the paper. The distance travelled by same compound with respect to the solvent is always constant. Thus the ratio of the distance that the compound travelled and the distance that the solvent travelled is denoted as R_f.

Summary

- Paper chromatography is an chromatography technique used to separate mixture of chemical substances into its individual compounds.
- Paper chromatography consists of two phases: one mobile phase and one contiguous stationery phase.
- Paper used in paper chromatography is made of cellulose.
- A suitable solvent (mobile phase) is moved along with a compound mixture through the paper according to the polarity and the degree of adhesion of each component on the stationery phase.
- The ratio of the distance that the compound travelled and the distance that the solvent travelled is denoted as R_f.

Paper Chromatography Applications

There are various applications of paper chromatography. Some of the uses of Paper Chromatography in different fields are discussed below:

- To study the process of fermentation and ripening.

- To check the purity of pharmaceuticals.
- To inspect cosmetics.
- To detect the adulterants.
- To detect the contaminants in drinks and foods.
- To examine the reaction mixtures in biochemical laboratories.
- To determine dopes and drugs in humans and animals.

Types of paper chromatography:

1. Ascending Paper Chromatography – The techniques goes with its name as the solvent moves in an upward direction.
2. Descending Paper Chromatography – The movement of the flow of solvent due to gravitational pull and capillary action is downwards hence the name descending paper chromatography.
3. Ascending – Descending Paper Chromatography – In this version of paper chromatography movement of solvent occurs in two directions after a particular point. Initially, the solvent travels upwards on the paper which is folded over a rod and after crossing the rod it continues with its travel in the downward direction.
4. Radial or Circular Paper Chromatography – The sample is deposited at the center of the circular filter paper. Once the spot is dried, the filter paper is tied horizontally on a Petri dish which contains the solvent.
5. Two Dimensional Paper Chromatography – Substances which have the same r_f values can be resolved with the help of two-dimensional paper chromatography.

Difference between Column Chromatography and Thin-Layer Chromatography
Now that we have looked at two types of chromatography, let us try to understand the basic differences between the two.

1) Thin-layer chromatography requires higher samples and lower analysis time. This is because the number of samples separated via TLC is much more than that done in the column chromatography process.
2) In Thin-layer chromatography, one makes use of strong reagents to identify the different components of the mixture. This works out well in comparison to column chromatography because the TLC plate can withstand strong solvents and colour creating agents.
3) The plates used in the thin-layer chromatography process are designed in such a manner that they can be heated to very high temperatures without undergoing any damage. This may not work out for Column chromatography plates.

4) Thin-layer chromatography is said to be more sensitive as there is less spreading of substance zones in this process in comparison to the column chromatography process.

Difference between Paper Chromatography and Thin layer chromatography

Properties	**Paper chromatography**	**Thin layer chromatography**
Principle	Partition chromatography	Absorption chromatography
Preparation time	Less	Comparatively more
Stationary phase	Water present in the pores of cellulose	Glass coated with silica gel
Mobile phase	Hydrophilic mobile phase: Isopropanol: Ammonia: Water Methanol: Water Hydrophobic mobile phase: Dimethyl ether: Cyclohexane Kerosene: Isopropanol	Pyridine, carbon-tetrachloride, acetone, glycerol etc.
Sample requirement	Less amount of sample is required	Comparatively requires little more sample
Heat treatment	Paper cannot be heated in an oven for long time	TLC plate can be heated in an oven for long time
Use of silica gel	It does not use silica gel	It makes the use of silica gel
Separation efficiency	More efficient for polar water soluble compounds.	Efficient for less polar compounds
Physical separation	Physical separation is found and ascending technique is preferred	It lacks the physical separation and descending technique is preferred
Use of corrosive reagents	Can’t use	It can be coated with corrosive reagents
Evaluation	Cannot evaluate under the UV-light	Spots can be evaluate under the UV-light
Time	It takes less time for the particle separation	It takes more time for the particle separation
Cost	Cheap	Comparatively costly