What is Chromatography?

In 1901, the Russian botanist, Mikhail Tswett, invented adsorption chromatography during his research on plant pigments. He separated different colored chlorophyll and carotenoid pigments of leaves by passing an extract of the leaves through a column of calcium carbonate, alumina, and sucrose, eluting them with petroleum ether/ethanol mixtures.

In Greek language, *chroma* means "color" and *graphos* means "to write".

According to IUPAC definition: "Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase), while the other (the mobile phase) moves in a definite direction"

Classification of chromatographic method:

The mobile phase can be a gas or a liquid, whereas the stationary phase can only be a liquid or a solid. When the stationary phase is contained in a column, the term column chromatography applies. The stationary phase can also occupy a plane surface, such as filter paper. This is called planar chromatography and includes thin-layer and paper chromatography and electrophoresis. Column chromatography can be subdivided into gas chromatography (GC) and liquid chromatography (LC) to reflect the physical state of the mobile phase. If the sample passing through the chromatograph is in the form of a gas, the analytical technique is known as gas chromatography.

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Gas chromatography comprises gas-liquid chromatography (GLC) and gas-solid chromatography (GSC), names that denote the nature of the stationary phase. Liquid-column chromatography embraces several distinct types of interaction between the liquid mobile phase and the various stationary phases. When the separation involves predominantly a simple partition between two immiscible liquid phases, one stationary and one mobile, the process is called liquid–liquid chromatography (LLC). In liquid–solid (or adsorption) chromatography (LSC) physical surface forces are mainly involved in the retentive ability of the stationary phase. Ionic or charged species are separated in ion chromatography (IC) by selective exchange with counterions of the stationary phase; this may be by ion-exchange chromatography (IEC), ion-pair chromatography, or ion exclusion chromatography. In

columns filled with porous polymers, components may be separated by exclusion chromatography (EC) [also called gel-permeation chromatography (GPC)]; separation is based largely on molecular size and geometry.

Mechanism of Separation/ Retention: Chromatographic processes can be classified according to the type of equilibration process or retention mechanism involved, which is governed by the type of stationary phase. Various bases of equilibration are: (1) adsorption, (2) partition, (3) ion exchange, and (4) size dependent pore penetration.

Partition Chromatography: The stationary phase of partition chromatography is usually a liquid supported on a solid or a network of molecules, which functions virtually as a liquid, bonded on the solid support. Again, the mobile phase may be a liquid (liquid–liquid partition chromatography) or a gas (gas–liquid chromatography, GLC). In the normal mode of operations of liquid–liquid partition chromatography, a polar stationary phase (e.g., cyano groups bonded on silica gel) is used, with a nonpolar mobile phase (e.g., hexane). When analytes (dissolved in the mobile phase) are introduced into the system, retention increases with increasing polarity. This is called normal-phase chromatography. If a nonpolar stationary phase is used with a polar mobile phase, the retention of solutes decreases with increasing polarity. This mode of operation is termed reversed-phase chromatography and is presently the most widely used mode. "Normal-phase" chromatography significantly predates the reversed-phase mode, and was originally called liquid chromatography. Only after "reversed-phase" chromatography came along, the need arose to distinguish between the two, and the older version, still more prevalent then, was termed "normal-phase."

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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. Thin-layer chromatography (TLC) is a special example of adsorption chromatography in which the stationary phase is planar, in the form of a solid supported on an inert plate, and the mobile phase is a liquid.

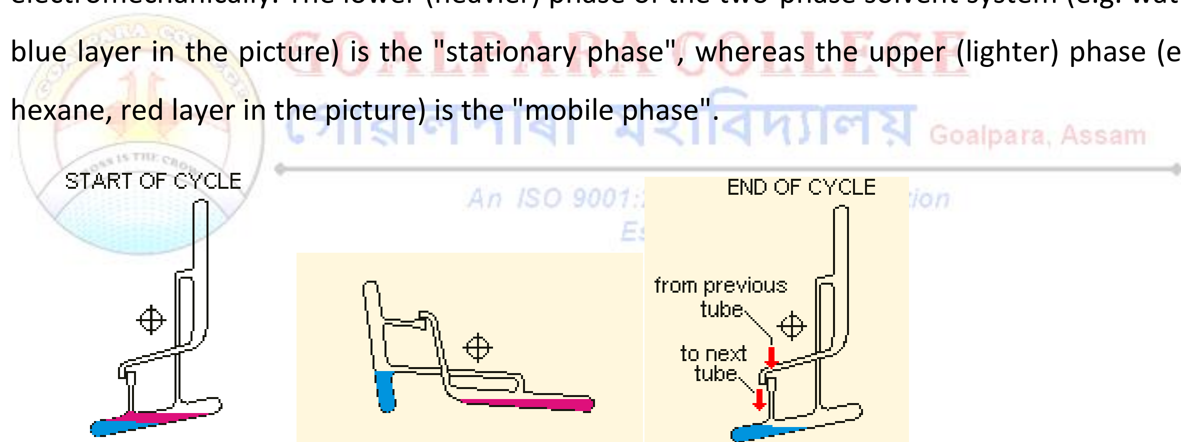
Ion-exchange and Size-exclusion Chromatography: Ion exchange chromatography uses supports with ion exchange functionalities as the stationary phase. The mechanism of separation is based on ion exchange equilibria. A reversible exchange of ions is possible

betweens ions of liquid phase (mobile phase) and a stationary solid phase (an insoluble substance having ionic sites). Hydrophobic interactions play a strong role in most ion exchange separations nevertheless, particularly in anion exchange chromatography.

In size exclusion chromatography, solvated molecules are separated according to their size by their ability to penetrate into porous pockets and passages in the stationary phase.

Counter-current Extraction - Craig Apparatus.

A method of multiple liquid-liquid extractions is countercurrent extraction, which permits the separation of substances with different distribution coefficients (ratios). A clever design known as Craig apparatus is used for this purpose (Lyman C. Craig, 1943). Craig apparatus consists of a series of glass tubes (r: 0, 1, 2..) that are designed and arranged such that the lighter liquid phase is transferred from one tube to the next. The liquid-liquid extractions are taking place simultaneously in all tubes of the apparatus which is usually driven electromechanically. The lower (heavier) phase of the two-phase solvent system (e.g. water, blue layer in the picture) is the "stationary phase", whereas the upper (lighter) phase (e.g. hexane, red layer in the picture) is the "mobile phase".



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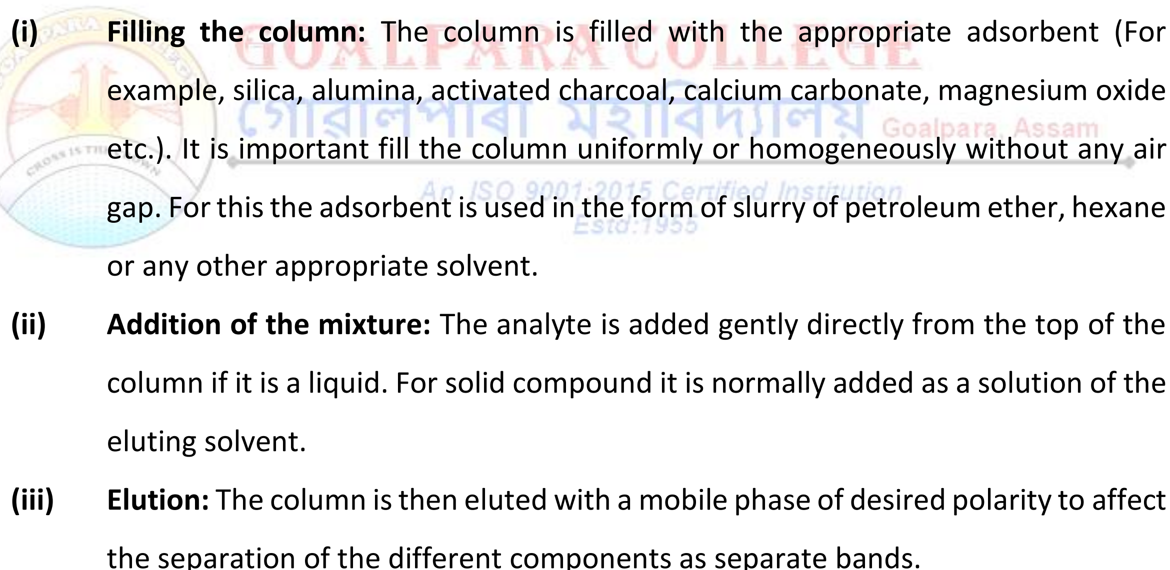
In the beginning, tube #0 contains the mixture of substances to be separated in the heavier solvent and all the other tubes contain equal volumes of the same solvent. The lighter solvent is added to tube #0, extraction (equilibration) takes place and the phases are allowed to separate. The upper phase of tube #0 is then transferred to tube #1 and fresh solvent is added to tube #0, and the phases are equilibrated again. The upper layers of tubes #0 and #1 are simultaneously transferred to tubes #1 and #2 respectively. This cycle is repeated to carry on the process through the other tubes of the apparatus. Obviously, substances with higher distribution ratio move faster than those with a lower distribution ratio.

Let us imagine that all of the tubes have V mL of water in them. We add a unit amount of solute to tube 0, followed by V mL of an organic solvent and shake up tube 0 to perform an extraction. After the extraction, let the fraction of the total solute mass s in the aqueous phase be a and that in the organic phase be b (a + b = 1). In the chromatographic context, we typically refer to the distribution constant (K_D in Equation 18.1), as the partition constant and denote it by K.

$$K = \frac{[S]_o}{[S]_a} = \frac{\frac{b}{V}}{\frac{a}{V}} = \frac{b}{a}$$

Column Chromatography: This type of chromatography is based on the principle that different compounds get adsorbed to different extent in a particular adsorbent (i.e. the solid stationary phase).

Procedure:



(i) **Filling the column:** The column is filled with the appropriate adsorbent (For example, silica, alumina, activated charcoal, calcium carbonate, magnesium oxide etc.). It is important fill the column uniformly or homogeneously without any air gap. For this the adsorbent is used in the form of slurry of petroleum ether, hexane or any other appropriate solvent.

(ii) **Addition of the mixture:** The analyte is added gently directly from the top of the column if it is a liquid. For solid compound it is normally added as a solution of the eluting solvent.

(iii) **Elution:** The column is then eluted with a mobile phase of desired polarity to affect the separation of the different components as separate bands.

Mechanism: As the solute and the solvent molecules move down the column, a competition sets in between them for adsorption on the adsorption sites of the adsorbent. Ultimately a dynamic equilibrium is set up at the interface where the solute and the solvent molecules get attracted, settle for a moment and leave the solid surface and re-enter the mobile liquid phase. In this competition of desorption and adsorption, the desorbed molecules having lesser affinity for solid surface (stationary phase) flow into the mobile layer faster than the desorbed molecules having greater affinity for solid surface.

Principles of Chromatographic Separations

In the case of chromatography, partition constant can be expressed as

$K = \frac{[S]_s}{[S]_m}$; $[S]_s$ = concentration of the analyte in stationary phase and $[S]_m$ = concentration of the analyte in mobile phase.

While the mechanisms of retention for various types of chromatography differ, they are all based on the dynamic distribution of an analyte between a fixed stationary phase and a flowing mobile phase. Each analyte will have a certain affinity for each phase. A small volume of sample is placed at the top of the column, which is filled with particles constituting the stationary phase and the solvent. Rather than an equilibrium-based "plate view" of chromatography, many holds that a "rate view" of chromatography to be more rigorous: in this view, the partition ratio is simply the ratio of the time a solute spends in the stationary phase to that it spends in the mobile phase. More solvent, functioning as mobile phase, is added to the top of the column and percolates through the column. The individual components interact with the stationary phase to different degrees, and the distribution is given in terms of partition constant. The distribution of the analyte between the two phases is governed by many factors: the temperature, the type of compound, and the stationary and mobile phases. solutes with a large K value will be retained more strongly by the stationary phase than those with a small K value. The result is that the latter will move along the column (be eluted) more rapidly.

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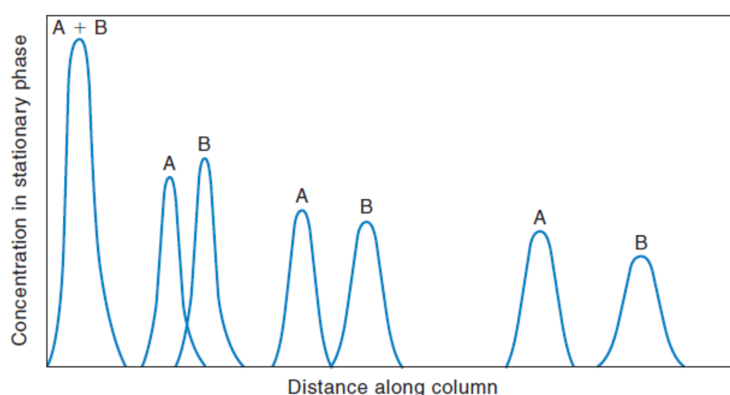

Figure. Distribution of two substances, A and B, along a chromatographic column in a typical chromatographic separation.

Theory of Column Efficiency in Chromatography

The separation efficiency of a column can be expressed in terms of the number of theoretical plates in the column. The more the number of plates, the more efficient is the column. The plate height, H, is the length of a column, L, divided by the number of theoretical plates, N:

$$H = \frac{L}{N}$$

The efficiency of a column is a function of several parameters. These include the size of the column packing particles, the uniformity of the packing, the flow of eluent, and the rapidity with which equilibrium is established between the two phases.

Reduced plate height

Reduced plate height, h, obtained by dividing by the particle diameter, d_p:

$$h = \frac{H}{d_p}$$

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For open tubular columns:

$$h = \frac{H}{d_c}$$

where d_c is the inner diameter of the column.

Paper Chromatography:

Paper chromatography is a planar chromatography which is mostly based on the principle of partition. In paper chromatography the substrate is a piece of porous paper with water adsorbed on it. The sample is placed on the paper as a spot or streak and then irrigated by the solvent system that percolates within the porous structure of the paper. Usually development of the chromatogram is stopped before the mobile phase reaches the farther edge of the paper, so the solute zones are distributed in space instead of time. The major limitations of paper chromatography are relatively long development times and less sharply defined zones as compared to thin-layer techniques.

Chromatography on paper is essentially a liquid–liquid partition in which the paper serves as carrier for the solvent system. Aqueous systems are used for strongly polar or ionic solutes.

Water is held stationary on the paper as a “water–cellulose complex” or puddles of water, organized and dense near the amorphous regions of the cellulose chains. The stationary phase is attained by exposing the suspended paper to an atmosphere saturated with water vapor in a closed chamber. If an aqueous buffer or salt solution is to be used as the stationary phase, the paper is drawn through the solution, allowed to dry, and then exposed to the atmosphere saturated with water vapor.

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