

# CHROMATOGRAPHY

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## Introduction to chromatography:

Chromatography is a non-destructive procedure for resolving a multi-component mixture of trace, minor, or major constituents into its individual fractions. Different variations may be applied to solids, liquids, and gases. While chromatography may be applied both quantitatively, it is primarily a separation tools.

Chromatography is relatively a new technique which was first invented by M. Tswett, a botanist in 1906 in Warsaw. In that year, he was successful in doing the separation of chlorophyll, xanthophyll and several other colored substances by percolating vegetable extracts through a column of calcium carbonate. the calcium carbonate column acted as an adsorbent and the different substances got adsorbed to different extent and this gives rise to coloured bands at different positions, on the column. Tswett termed this system of coloured bands as the *chromatogram* and the method as *chromatography* after the Greek words *chroma* and *graphs* meaning “colour” and “writing” respectively. However, in the majority of chromatographic procedures no coloured products are formed and the term is a misnomer.

Considerable advances have since been made and the method is used to separate coloured as well as colourless substances. The column of calcium carbonate, used in Tswett’s method, remains stationary and is therefore termed as the stationary phase. The solution of vegetable extracts moves or flows down the column and is therefore termed as the mobile phase. Chromatography may be regarded as a method of separation in which separation of solutes occur between a stationary phase and a mobile phase.

## **Definition of chromatography**

Chromatography may be defined as a method of separating a mixture of components into individual components through equilibrium distribution between two phases.

Essentially, the technique of chromatography is based on the differences in the rate at which the components of a mixture move through a porous medium (called stationary phase) under the influence of some solvent or gas (called moving phase).

**The chromatography method of separation, in general, involves the following steps :**

1. Adsorption or retention of a substance or separation, in general involves the following steps:
2. Separation of the adsorbed substances by the mobile phase.
3. Recovery of the separated substances by a continuous flow of the mobile phase, the method being called elution.
4. Qualitative and quantities analysis of the eluted substances.

**Types of chromatography**

In chromatography, the stationary phase may be a solid or a liquid and the mobile phase may be liquid or a gas. Depending on the stationary and the mobile phase used, separation occurs because of a combination of two or more factors such as rates of migration, capillary action, extent of adsorption etc., chromatographic methods can be classified on the basis of the stationary and the mobile phase used.

**The various types are tabulated in table**

<b>Technique</b>	<b>Stationary Phase</b>	<b>Mobile phase</b>
1. Column chromatography or adsorption chromatography	Solid Liquid	Liquid Liquid
2. partition chromatography	Liquid	Liquid
3. paper chromatography	Liquid or	Liquid
4. thin layer chromatography (TLC)	solid	Gas
5. Gas-liquid chromatography (GLC)	Liquid	Gas
6. Gas-solid chromatography (GSC)	Solid	Liquid
7. Ion exchange chromatography	Solid	

While the above classification is based on the phases involved there are a great number of combinations of phases and processes giving rise to a large number of methods with individual names (table)

**Table : classification of chromatographic methods**

Stationary phase	Mobile phase	Name
Solid	Liquid	Plane chromatography
		Paper chromatography (PC)
		Thin layer chromatography (TLC)
		Adsorption column chromatography
		High performance liquid chromatography (HPLC)
Solid (ion exchange resin)	Liquid	Ion exchange chromatography (IEC)
Solid	Gas	Gas-solid chromatography (GSC)
Solid matrix	Liquid	Gel Permeation chromatography (Exclusion chromatography) (GPC)
Liquid	Gas	Gas-liquid chromatography (GLC)
Liquid	Liquid	Liquid –liquid chromatography (LLC)

The method to be discussed in the following chapters will be in terms of the terminology commonly employed and will come under one or the other of the categories listed in the table.

A more recent development in liquid-liquid chromatography is Countercurrent Chromatography which entirely eliminated the use of a solid matrix support. Another form of chromatography where the stationary phase is a porous gel and the separation is according to the size of the molecule is Gel (exclusion) chromatography. Chromatography using gels modified to develop highly specific biochemical reactions for separations is termed as Affinity chromatography (also called bioaffinity chromatography). Other modifications of this technique are metal–Chealte Chromatography, Ligand Exchange Chromatography, and Dye-Ligand Affinity Chromatography. A newer technique which makes use of all the above principles permits very rapid separations is High Performance Liquid Chromatography (HPLC).