INTRODUCTION TO CHROMATOGRAPHY

What is Chromatography?

- **Word:** Derived from the Greek word ***Chroma*** meaning color, **graphein** meaning write.
- **Work:** Chromatography provides a way to identify unknown compounds and separate mixtures

Purpose of Chromatography

- **Analytical**

-Determine chemical composition of a sample (E.g. Fatty acid composition of fats and oils…)

- **Preparative**

-Used to purify sufficient amount of sample (E.g. Purifying polar compounds from apolar ones, free fatty acids from triglycerides), i.e.preparative HPLC or column chromatography

Applications of Chromatography

- **Chromatography is a technique for**
  - separating compounds from the mixtures
  - identifying unknown compounds
  - increasing the purity or concentration of compounds
  - monitoring formation or decomposition fo the compouds during the reactions

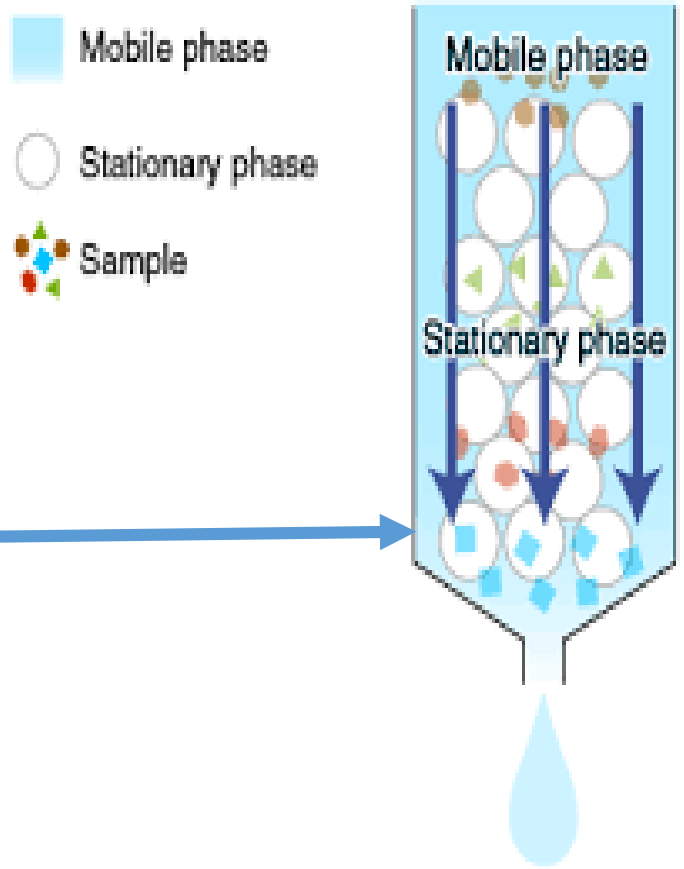
Definitions

Chromatography: A separation technique based on the different interactions of compounds with two phases, a *mobile phase* and a *stationary phase*, as the compounds travel through a supporting medium

mobile phase: The phase carrying out the sample in the column (Gas or liquid)

stationary phase: a layer or coating on the supporting medium that interacts with the analytes (compounds)

supporting medium: a solid surface on which the stationary phase is bound or coated



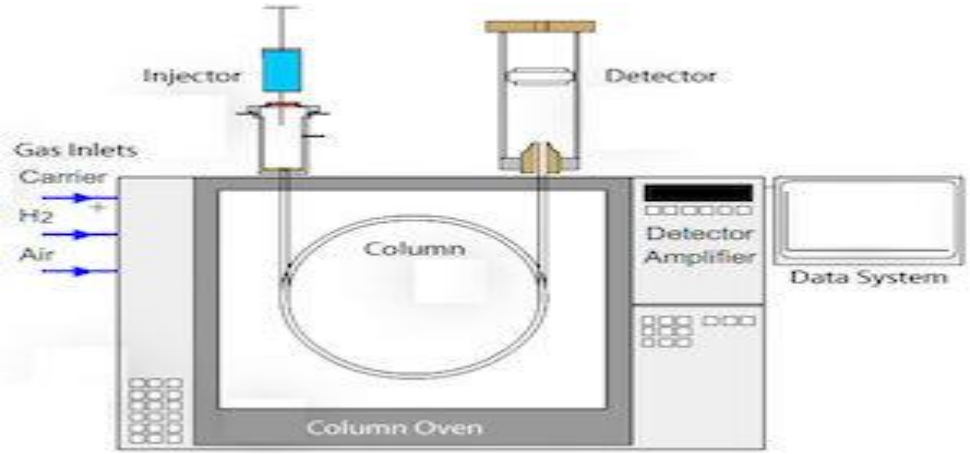
Definitions cont…….

- Isocratic: Mobile phase with same composition
- Gradient: Mobile phase with various composition
- Partition: Interaction of a solute (compound) with mobile and stationary phase
- Elution: Passing a compound through stationary phase, depending on relative affinities between analytes and support
- Retention time: Time for a compound to pass stationary phase

How Does Chromatography Work?

In all chromatographic separations, the sample is transported or injected in a **mobile phase**. The mobile phase can be a gas, a liquid, or a supercritical fluid.

The mobile phase is then forced through a **stationary phase** held in a column or on a solid surface.

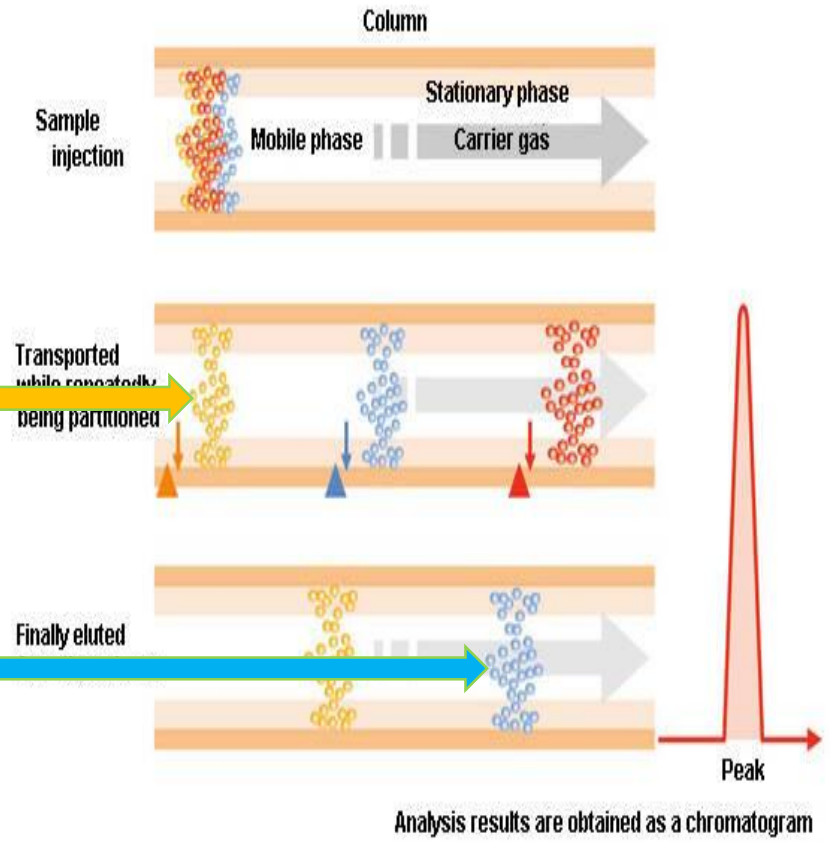


How Does Chromatography Work?

The **sample** then has the **opportunity to interact** with the stationary phase as it moves.
Samples that interact greatly, then appear to move more slowly.

Samples that interact weakly, then appear to move more quickly.

Because of this difference in rates, the samples can then be **separated into their components**.

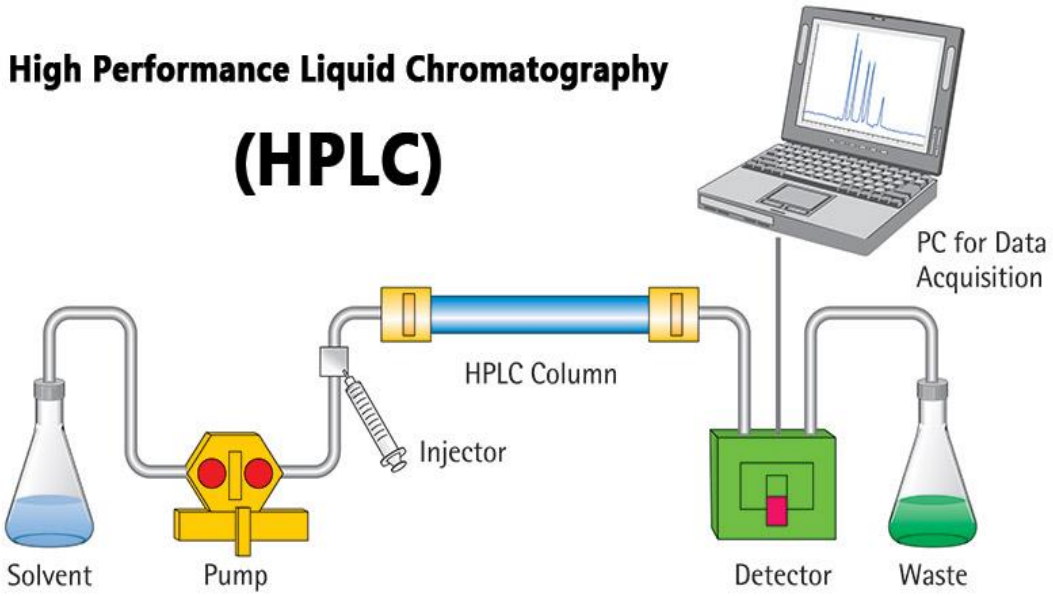


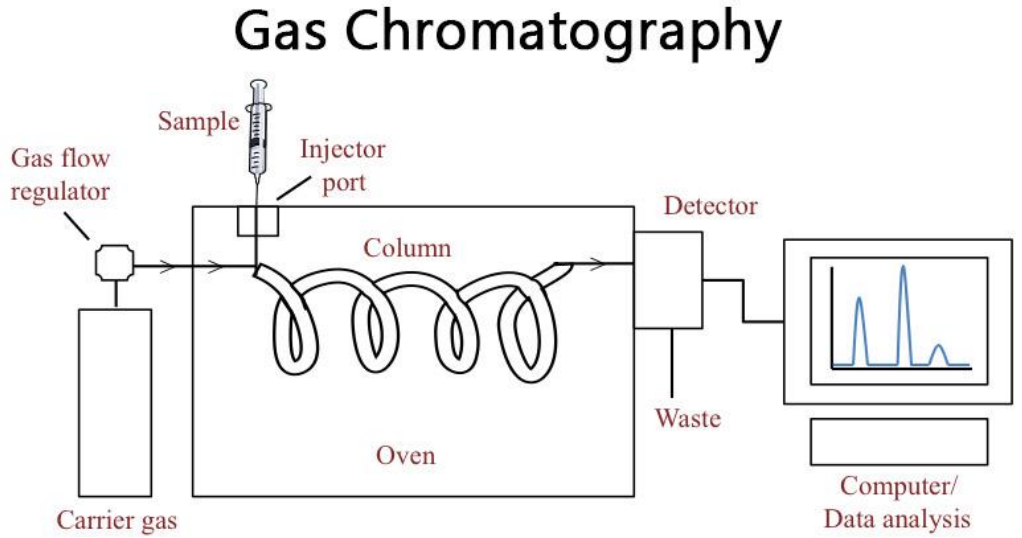
Types of Chromatography

Classification of chromatography according to mobile phase:

1- Liquid chromatography: mobile phase is a liquid (HPLC).

2- Gas chromatography : mobile phase is a gas (GC).





Classification according to the packing of the stationary phase:

1- Thin layer chromatography (TLC): the stationary phase is coated as a thin layer (silica) on the glass, plastic or aluminium plates.

2- Paper chromatography (PC): the stationary phase is a thin film of liquid supported on an inert support.

3- Column chromatography (CC): stationary phase (like silica or alumina) is packed in a glass column.