Introduction to Chromatography

Chromatographic processes can be defined as separation techniques involving mass transfer between stationary and mobile phases.

**stationary phase** - common name for the column packing material in any type of chromatography.

**mobile phase** - liquid media that continuously flows through the column and carries the **analytes**.

**analyte** - the chemical species being investigated (detected and quantitated) by an analytical method.
High Performance Liquid Chromatography (HPLC)

HPLC utilizes a liquid mobile phase to separate the components of a mixture. These analytes are first dissolved in a solvent, and then forced to flow through a chromatographic column under a high pressure. In the column, the mixture is resolved into its components. The amount of resolution is important, and is dependent upon the extent of interaction between the solute components and the stationary phase.
Types of HPLC

In **adsorption chromatography** the stationary phase is an adsorbent (like silica gel or any other silica-based packing) and the separation is based on repeated adsorption-desorption steps.

In **normal-phase chromatography**, the stationary bed is strongly polar in nature (e.g., silica gel), and the mobile phase is nonpolar (such as n-hexane or tetrahydrofuran). Polar samples are thus retained on the polar surface of the column packing longer than less polar materials.

In **reversed-phase chromatography** the stationary bed is nonpolar (hydrophobic) in nature, whereas the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. Here the more nonpolar the material is, the longer it will be retained.
In **size exclusion chromatography** the column is filled with material having precisely controlled pore sizes, and the sample is simply sieved or filtered according to its solvated molecular size. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the pores of the packing particles and elute later.

For historical reasons, this technique is also called **gel permeation chromatography** although, today, the stationary phase is not restricted to a "gel."
In **ion-exchange chromatography** the stationary bed has a charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionizable samples. The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time.
Eluent polarity plays the most important role in all types of HPLC.

There are two elution types:

In an **isocratic elution**, the eluent composition remains constant as it is pumped through the column during the whole analysis.

In a **gradient elution**, the eluent composition (and strength) is steadily changed during the run.
Parametrizing Retention in HPLC

The easiest way to characterize the chromatographic retention is to measure the time between injection and the maximum of the detector response for the analyte. This parameter, which is usually called the retention time $t_R$, is inversely proportional to the eluent flow rate.

The product of the retention time and the eluent flow rate is called the retention volume $V_R$ and it represents the volume of the eluent passed through the column while eluting a particular analyte.
Component retention volume $V_R$ can be divided into two parts:

1. **Reduced retention volume**, which is the volume of the eluent that passed through the column while the component was stuck to the surface.

2. **Dead volume**, which is the volume of the eluent that passed through the column while the component was moving with the liquid phase.

The dead volume $V_o$ is equal to the volume of the liquid phase in the column, and it will be the same for any component eluted on this column.
The more universal and fundamental retention parameter is the ratio of the retention volume to the dead volume:

\[ k = \frac{V_R}{V_o} \]

Historically, a slightly different retention parameter, called capacity factor \( k' \) was introduced:

\[ k' = \frac{V_R - V_o}{V_o} \]
Band broadening (column efficiency)

After injection, a narrow chromatographic band is broadened during its movement through the column. The higher the column band broadening, the smaller the number of components that can be separated in a given time. In other words, the sharpness of the peak is an indication of how good, or efficient a column is.
The peak width is an indication of peak sharpness and, in general, an indication of the column efficiency. However, the peak width is dependent on a number of parameters (column length, flow rate, particle size). Flow rate is the only parameter that can be changed from run to run on the same column.

In absence of the specific interactions or sample overloading, the chromatographic peak can be represented by a gaussian curve with the standard deviation $\sigma$. The ratio of standard deviation to the peak retention time $\sigma / t_R$ is called the relative standard deviation, which is independent of the flow rate.
The convention today is to describe the efficiency of a chromatographic column in terms of the **plate number** N, defined by

\[ N = \left( \frac{t_R}{\sigma} \right)^2 \]

In practice, it is more convenient to measure peak width either at the base line, or at the half height, and not at 0.609 of the peak height, which actually correspond to 2 \( \sigma \).

\[ N = 16 \frac{t_R^2}{w^2_{\text{base}}} \quad N = 5.545 \frac{t_R^2}{w^2_{1/2}} \]

The plate number depends on column length: the longer the column, the larger the plate number. Therefore, the **plate height** term has been introduced to measure how efficiently the column has been packed, \( h = \frac{L}{N} \)
Selectivity and Resolution

Values measured from a chromatogram containing two peaks
Selectivity is the ratio of the capacity factors of both peaks.

\[
\alpha = \frac{V_{R,1} - V_o}{V_{R,2} - V_o} = \frac{k'_1}{k'_2}
\]

This parameter is independent of the column efficiency; it only depends on the nature of the components, eluent type, eluent composition, and adsorbent surface chemistry. In general, if the selectivity of two components is equal to 1, then there is no way to separate them by improving the column efficiency.
Band broadening theory (Van Deemter equation)

It is well recognized now that column band broadening originates from three main sources:

1. multiple path of an analyte through the column packing;
2. molecular diffusion;
3. effect of mass transfer between phases.

In 1956 J.J. Van Deemter introduced the equation which combined all three sources and represented them as the dependence of the theoretical plate height (HETP) on the mobile phase linear velocity.
Band broadening is caused by differing flow velocities through the column, which may be written in form

\[ H_p = 2\lambda d_p \]

where \( H_p \) is the HETP arising from the variation in the zone flow velocity, \( d_p \) is the particle diameter (average), and \( \lambda \) is a constant that is close to unity.
The longitudinal diffusion (along the column long axis) leads to band broadening of the chromatographic zone. This process may be described by equation:

\[ H_d = 2 \frac{\gamma D_m}{v} \]

where \( D_m \) is the analyte diffusion coefficient in the mobile phase, \( \gamma \) is the factor that is related to the diffusion restriction by column packing, and \( v \) is the flow velocity.

It is obvious from the above equation that the higher the eluent velocity, the lower the diffusion effect on the band broadening. Molecular diffusion in the liquid phase is about five orders of magnitude lower than that in the gas phase, thus this effect is almost negligible at the standard HPLC flow rates.
where: $d_p$ is the particle diameter, $D_m$ is the diffusion coefficient of the analyte in the mobile phase, $\omega$ is the coefficient determined by the pore size distribution, shape, and also particle size distribution, and $v$ is the flow velocity.

The above equation describes the linear dependence of HETP on the flow rate. The slower the velocity, the more uniformly analyte molecules may penetrate inside the particle, and the less the effect of different penetration on the efficiency. On the other hand, at the faster flow rates the elution distance between molecule with different penetration depth will be high.
The most significant result is that we can find an optimum eluent flow rate where the column efficiency will be the best.