VII.1.12 Kinetic theory in chromatography:

Kinetic theory in chromatography explains how solutes separate based on different factors that influence the separation efficiency. The efficiency is measured in terms of the number of theoretical plates. The **Van Deemter, Knox, and Giddings equations** are mathematical models used to describe the effect of these factors on the equivalent height of a theoretical plate (HETP).

(a) Van Deemter equation :

- **Formula** :

$$H = A + B/u + C.u$$

$$B = 2\gamma D_M$$

Or :

- H : Equivalent height of a theoretical plate (HETP)
- A : Eddy term (turbulent diffusion)
- B : Longitudinal diffusion term
- C : Mass transfer term
- u : Linear velocity of the mobile phase: *reduced speed*
- γ ***tortuosity*** factor > 1
- D_M : Diffusion coefficient of the solute in the mobile phase

- **Explanation :**
  - ✓ **Term A (Eddy Diffusion):** Related to the multiple paths that molecules can take through the stationary phase particles.
  - ✓ **B/u term (Longitudinal diffusion):** More significant at low mobile phase speeds. Molecules diffuse along the column, which broadens the peaks.
  - ✓ **Term C · u (Mass transfer):** More important at high speeds. Mass transfer is the resistance to the movement of solutes between the mobile and stationary phases.

✓ **Application:** This equation helps determine the optimum mobile phase velocity that minimizes H and maximizes separation efficiency.

(b) Knox equation :

- **Formula:**

$$H = A' + B'.u^{0.5} + C'.u$$

- Where A', B' and C' are specific constants related to the nature of the column and the solutes.

✓ **Application:** The Knox equation is useful for modern high performance LC columns where mass transfer is a limiting factor at high speed.

(c) Giddings equation :

✓ **Formula:**

$$H = A + B/u + C_1 . u + C_2 . u^2$$

- Where C1 and C2 represent different mass transfer terms in the mobile and stationary phase.

- **Explanation :**

  - Adds an extra term ($C_2 \cdot u^2$) to represent the extra dispersion at very high speeds.
  - Takes into account the complex effects of mass transfer and molecular interactions under extreme conditions.

- **Application:** Useful for describing the behavior of solutes in columns using modern stationary phases or high viscosity mobile phases.

❖ **Application of kinetic theory in the analysis of chromatograms:**

- **Reading a chromatogram:** Using these equations, we can estimate the height of the peaks, their width at half-height, and evaluate the quality of separation.
  - ✓ HH H value indicates an efficient column with narrow peaks.
  - ✓ High efficiency translates into a high number of theoretical plateaus.
- ***Example:*** *When analyzing a complex mixture, optimizing the mobile phase velocity using the Van Deemter equation can reduce analysis time while maintaining good resolution.*

VII.1.13 Pressure loss in a column: (DARCY's law):

- **Pressure drop** in a chromatographic column is a decrease in pressure across the column as the mobile phase flows through the stationary phase. This pressure drop is essential to understand because it directly influences flow rate, analysis time, and separation efficiency.

- **Introduction to pressure loss**

In the context of chromatography, pressure drop refers to the pressure difference between the inlet and outlet of the column. This pressure drop is due to the resistance of the flow of the mobile phase through the stationary phase particles.

- **Principle of pressure loss (Darcy's law):**

Darcy's law is used to describe fluid flow in a porous medium, and it is applied to chromatographic columns to model pressure drop.

Darcy equation:

$$\Delta P = \frac{\eta L v}{K^0} \qquad \text{and} \qquad K^0 = \frac{d_p^2}{180} \times \frac{\varepsilon^3}{(1-\varepsilon)^2}$$

- ΔP : Pressure loss or pressure difference (Pa)
- η : Viscosity of the mobile phase (Pa.s)
- L : Length of the column (m)
- v : Linear velocity of the mobile phase (m/s)
- k^0 : Permeability of the stationary phase (m²)
- dp : Diameter of stationary phase particles (m)

- ***Viscosity (η)*** *: A measure of the resistance of the mobile phase to flow. Higher viscosity results in greater pressure drop.*
- ***Column length (L)*** *: The longer the column, the higher the pressure loss.*
- ***Linear velocity (v)*** *: The higher the velocity of the mobile phase, the greater the pressure drop.*
- ***Permeability (k)*** *: Measure of the ability of the stationary phase to allow the mobile phase to pass through. Low permeability (due to fine and dense particles) results in high pressure drop.*
- ***Particle diameter (dp)*** *: Smaller particle diameters increase contact area and flow resistance, which increases pressure loss.*

- **Relationship between pressure drop and separation efficiency:**

The pressure drop directly influences the flow rate and the maximum pressure that a column can support. If the pressure becomes too high:

- There may be deformation or rupture of the spine.
- The optimal flow rate of the mobile phase cannot be maintained, which affects the efficiency and resolution of chromatographic peaks.

- **Flow optimization:**
  - ✓ **Choice of stationary phase**: Using larger particles or stationary phases with greater permeability can reduce pressure drop.
  - ✓ **Mobile phase speed**: It is crucial to adjust the speed to avoid excessive pressure increase while maintaining good separation efficiency.
- **Practical application of Darcy's law in chromatography:**

In HPLC chromatography, pressure drop is a critical factor when choosing analysis parameters. For example:

- ✓ For rapid separation with high flow rate, short columns with larger particles are used to minimize pressure drop.
- ✓ In analyses requiring high resolution, finer stationary phase particles are used, which increases the pressure drop. In this case, the systems must be equipped with pumps capable of handling high pressures.

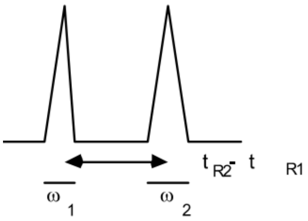
VII.1.14 optimizing the conditions of an analysis:

The idea is to obtain a high resolution in very short time:

- ✓ It is possible to consider increasing the length of the column (the analysis speed will increase as well as the pressure drop)
- ✓ The particle diameter can be reduced (HEPT decreases, but the pressure loss increases)
- ✓ Can be increased (HEPT increases the pressure too).

The resolution Rs between two peaks is defined by the relation:

$$R = 2 \times \left(\frac{t_{R1} - t_{R2}}{\varpi_1 + \varpi_2} \right)$$



- Resolution optimization:

It is necessary to increase Rs

$$\begin{cases} R_S = \dfrac{\sqrt{N}}{4} \times \dfrac{\alpha - 1}{\alpha} \times \dfrac{k_2'}{1 + k_2'} \\ R_S = \dfrac{\sqrt{N_{ef}}}{4} \times \dfrac{\alpha - 1}{\alpha} \qquad \text{with } N_{ef} = 16\left(\dfrac{t_R - t_0}{\varpi} \right)^2 \end{cases}$$

N_{ef} *number of effective trays*

- The peak capacity of a column is defined by:

$$n = 1 + \sqrt{N/16}\, \ln\left(\frac{t_0}{t_\alpha} \right)$$

✓ $(t_\alpha ; t_0)$Retention time of the first and last peak of the chromatogram, according to HERMAN (1985): Shown for n = 60, there is a 90% probability of separating 9 compounds.

VII.1.15 Optimization of Chromatographic Analysis Conditions:

Optimization of analytical conditions in chromatography is essential to obtain efficient separations, rapid analyses and reproducible results. This step aims to adjust various chromatographic parameters in order to improve resolution, reduce analysis time and ensure optimal sensitivity.

- **Objectives of optimization:**
  - ✓ **Improve Resolution (R)**: Effectively separate closely related compounds.
  - ✓ **Reduce analysis time (tR)**: Speed up the analytical process.
  - ✓ **Achieve maximum reproducibility**: Ensure consistent results across multiple analyses.
  - ✓ **Increase sensitivity**: Ensure accurate detection of low concentrations.

- **Parameters influencing optimization:**

a) **Mobile phase:**

✓ **Composition of the mobile phase:**

- In liquid chromatography (HPLC), vary the water/organic solvent ratio (acetonitrile, methanol, etc.) to adjust the polarity.
- In gas chromatography (GC), change the type of carrier gas (helium, nitrogen, hydrogen) or its flow rate.

- ✓ **pH and buffer concentration:** In liquid chromatography, pH influences the loading of analytes and the stationary phase. It must be adjusted to avoid degradation of sensitive compounds.

b) **Stationary phase:**

- ✓ **Nature of the stationary phase:** Adapt the type of stationary phase (polar, apolar, chiral, etc.) according to the polarity or chirality of the analytes.
- ✓ **Column dimensions:** A small diameter column and small particle size (in HPLC) increases efficiency, but also increases pressure.

c) **Mobile phase flow rate:** In liquid or gas chromatography, adjusting the flow rate reduces retention times and improves resolution. However, too high a flow rate can reduce efficiency (effect on the number of theoretical plates).

d) **Temperature :**

- ✓ In gas chromatography, high temperature reduces retention times, but may affect the stability of thermolabile analytes.
- ✓ In liquid chromatography, increasing the temperature can decrease the viscosity of the mobile phase, facilitating flow.

e) **Column length:** A longer column increases resolution, but at the cost of longer analysis time.

f) **Injection volume:** Too large an injection volume can saturate the column and reduce separation efficiency.

VII.1.16 Injectors/injection:

- **Definition:** An injector is an essential component of the HPLC system that allows the sample to be introduced into the mobile phase flow before it enters the column. It ensures precise and reproducible injection.
- **Principle:**

The injector introduces a controlled amount of sample into the moving mobile phase. This is done without disturbing the flow or pressure of the system. The sample is typically mixed with the mobile phase before passing into the column.

- **Different Types of Injectors:**
  - ✓ **Depending on the sample type:**
    - ✓ Liquid samples: conventional injectors.
    - ✓ Gas samples: specific injectors (in gas chromatography - GC).
  - ✓ **Depending on the type of chromatography:**
    - ✓ **Classic HPLC:** manual and automatic injectors.
    - ✓ **UHPLC chromatography:** high precision injectors suitable for high pressures.
  - ✓ **Common types of injectors:**
    - ✓ **Loop Injector:** Allows reproducible injection of a fixed volume.
    - ✓ **Automatic Injectors (Autosampler):** Automate injection for multiple samples, reducing human error.

✓ **Inline injectors:** Integrated into the mobile phase flow for continuous samples.

- **Injection Methodology:**
- Prepare a syringe or use an automatic sampler.
- Introduce the sample into the injection loop or directly into the system depending on the injector type.
- Activate the system so that the sample is carried by the mobile phase towards the column.

- **How to Inject the Sample:**

✓ **Amount of sample injected:**

- Depends on column capacity and sample concentration.
- Typically between 1 and 100 µL for standard HPLC columns.

✓ **Tips for an optimal injection:**

- Use a clean syringe without air bubbles.
- Ensure that the sample is homogeneous.
- Inject slowly to avoid disturbances in the system.

- **Sample Preparation:**

✓ **Filtration:** Use 0.22 µm or 0.45 µm filters to remove particles that could clog the column.

✓ **Dissolution:** Dissolve the sample in a solvent compatible with the mobile phase.

✓ **Balancing:** Check that the concentration is suitable to avoid saturations or interferences.

- **Sample Standards:**

✓ **Purity:** Samples must be free of contaminants that could affect results.

✓ **Compatibility:** The dissolution solvent must be compatible with the mobile phase to avoid precipitation or incompatible mixtures.

✓ **Concentration:** Adjusted according to analytical needs and detector detection limits.

- **Standards and Their Preparation:**

✓ **Definition:** Standards are reference substances used for instrument calibration.

✓ **Preparation :**

- Accurately weigh a known quantity of standard.
- Dissolve in a suitable solvent to obtain an exact concentration.

✓ **Standards:**

- Store standards under appropriate conditions to prevent degradation.
- Prepare standard solutions from the standard to calibrate the system.

VII.1.17 Mobile Phase:

- **Definition:** The mobile phase is the moving liquid in the HPLC system. It transports the analytes through the column and plays a crucial role in their separation.
- **Different Types of Mobile Phases:**
  - ✓ **According to polarity:**
    - **Polar:** Water, weak acids, buffers (used in normal phase).
    - **Nonpolar:** Organic solvents such as methanol or acetonitrile (used in reversed phase).
    - **Mixed:** Mixture of water and organic solvents to modulate polarity.
  - ✓ **Depending on the type of samples:**
    - ✓ Polar samples: non-polar mobile phases.
    - ✓ Nonpolar samples: polar mobile phases.
    - ✓ Biomolecules: Specific buffers to maintain the stability of molecules.
- **How to Adjust the Flow Rate of the Mobile Phase:**
  - ✓ Use a pump to adjust the flow rate.
  - ✓ The flow rate is expressed in mL/min and must be adapted to the column used (generally 0.5 to 2 mL/min).
  - ✓ Equilibrate the column with the mobile phase before sample injection.

VII.1.18 Pumps :

- **Role of Pumps:**
  - Maintain a constant and precise flow rate of mobile phase.
  - Withstand high pressures up to 600 bar.
- **Types of Pumps:**
  - **Isocratic pumps:** Use a single mobile phase composition.
  - **Gradient pumps:** Allow the composition of the mobile phase to be varied during analysis (used for complex mixtures).