VII.1.7 Columns/stationary phase/ Number of theoretical plates

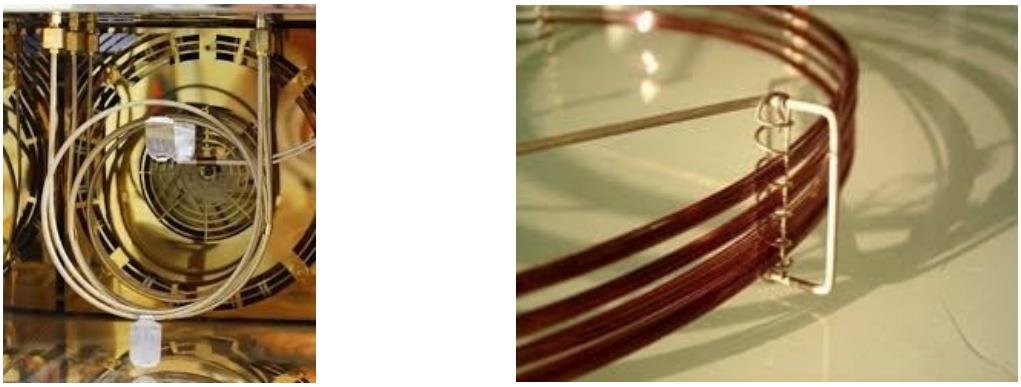

Fig V.6 : Chromatography columns

VII.1.7 1 Chromatographic column:

A chromatographic column is a cylindrical tube containing the **stationary phase** , through which the **mobile phase** transports the analytes. The column is essential for the separation of compounds in a mixture.

a) Column Types :

- **Packed columns** :
  - ✓ Used in liquid chromatography (HPLC) and gas chromatography (GC).
  - ✓ **stationary phase** particles or support material coated with stationary phase.
  - ✓ Typical internal diameter: 2-4 mm, length: 10-30 cm for HPLC.
- **Capillary columns (open tubular columns)** :
  - ✓ Mainly used in gas chromatography (GC).
  - ✓ Capillary tube with a thin layer of **stationary phase** on the inner wall.
  - ✓ Inner diameter: 0.1-0.5mm, length: 10-100m.

b) Column settings :

✓ **Column length** (L): Affects the number of theoretical plates and the resolution of the separation.

✓ **Internal Diameter** : The smaller the diameter, the better the resolution, but this may result in increased pressure.

✓ **Material Type** : Stainless steel, glass, or polymers, depending on analytical needs.

VII.1.7 2 Stationary Phase:

The **stationary phase** is the component of the column with which the analytes interact. It can be solid or liquid (supported by a solid) and determines the separation mechanism.

a) Types of Stationary Phases :

✓ **Polar stationary phase** :

- Capable of retaining polar compounds.

- Example: Silica (SiO_2), cyano (CN).

✓ **Nonpolar stationary phase** :

- Capable of retaining non-polar compounds.
- Example: C18 (octadecylsilane, ODS), C8.

✓ **Chiral stationary phase** :

- Used to separate enantiomers.
- Composed of materials that interact differently with each enantiomer.

b) Choice of Stationary Phase :

✓ Depends on the nature of the analytes and the purpose of the separation.

✓ A nonpolar stationary phase is chosen for nonpolar compounds, and a polar phase for polar compounds.

VII.1.7 3 Number of Theoretical Plates (N):

The **number of theoretical plates** is a measure of the efficiency of the chromatographic column. It reflects the degree of separation that the column can achieve.

a) Definition :

- The number of theoretical plates (N) indicates the number of equilibrium zones in a column where the analyte distributes between the mobile phase and the stationary phase.
- The higher the N , the more efficient the separation and the narrower the chromatographic peaks.

b) Calculation formula:

The number of theoretical plates is calculated by:

$$N = 16\left(\frac{t_R}{\varpi}\right)^2 = 5.54\left(\frac{t_R}{\delta}\right)^2$$

c) Equivalent Height of a Theoretical Plateau (HEPT or H):

HEPT **or H is another measure** of column efficiency, given by:

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

➢ **L** : Length of the column.

➢ The smaller the value of H , the better the efficiency of the column.

d) Factors influencing N and H:

✓ **Particle size** : Smaller particles increase N .

✓ **Column length** : A longer column increases N , but may require higher pressure.

✓ **Mobile phase flow rate** : An optimal flow rate maximizes N .

e) Applications of the Number of Theoretical Plateaus:

✓ **Performance evaluation** : N is used to compare the efficiency of the columns.

- ✓ **Separation optimization** : Column parameters can be adjusted to maximize N
- ✓ **Chromatographic peak quality : A high** NN N value corresponds to narrower and better defined peaks.

- ***Application Example: Separation of Aniline and Toluene:***

Suppose we are analyzing a mixture of aniline and toluene. The chromatogram shows two distinct peaks, with:

- ➢ ***Retention time*** *for aniline* t_R *=5.2 min.*
- ➢ ***Retention time*** *for toluene* t_R *7.8 min.*
- ➢ ***Half-height width*** *of aniline* $\omega_{1/2}$ *= 0.12 min.*
- ➢ ***Half-height width*** *of toluene* $\omega_{1/2}$ *= 0.15 min.*
- ➢ *By applying the formula N*

Conclusion: Understanding column parameters, stationary phase, and number of theoretical plates is crucial to optimizing a chromatographic method. Column efficiency can be adjusted by changing parameters such as particle size, column length, and mobile phase flow rate to achieve optimal separations and accurate analytical results.

f) **Symmetry Factor :**

- **Definition :**

The symmetry factor (or asymmetry factor) is a measure of the shape of a chromatographic peak. Ideally, a chromatographic peak should be symmetrical and Gaussian-shaped, but in practice, peaks can be asymmetrical. The symmetry factor (As) is used to quantify this deviation from ideal symmetry.

- **Formula :**

The symmetry factor is given by:

$$A_S = \frac{b}{a}$$

- ✓ **a** : Distance from peak start to left inflection point (measured at 10% of peak height).
- ✓ **b** : Distance from the end of the peak to the right inflection point (measured at 10% of the peak height).

- **Interpretation :**
  - ✓ **As = 1** : The peak is perfectly symmetrical.
  - ✓ **As > 1** : The peak is spread to the right (peak tail or "tailing").
  - ✓ **As<1** : The peak is spread to the left (peak front or "fronting").
- **Importance :**

- **Optimization** : A symmetry factor close to 1 is desirable for good separation and accurate quantification of analytes.
- **Diagnosis** : An asymmetric peak may indicate chromatographic column problems, such as sample overload or unwanted interactions with the stationary phase.

- ***Examples of use :***

In HPLC and GC chromatography, the symmetry factor is used to assess peak quality. High symmetry factors (e.g. As > 2) may require optimization of chromatographic conditions or column cleaning.

(g) Capacity Factor (or Retention Factor) in Chromatography:

The capacity factor, also called retention factor (symbolized by **k'**), is a fundamental measurement used in chromatography to describe the behavior of an analyte during separation. It indicates how much time a molecule spends in the stationary phase relative to the mobile phase. It is an essential parameter for evaluating the efficiency of the separation.

- Definition: The capacity factor **k'** is defined as the ratio of the time a solute spends in the stationary phase to the time it spends in the mobile phase. It is also interpreted as the number of times a molecule interacts with the stationary phase before being eluted: The formula for the capacity factor is given by:

$$k' = \frac{t_R - t_0}{t_0}$$

- ✓ $\mathbf{t_R}$: Solute retention time (time taken for the solute to be eluted from the column).
- ✓ $\mathbf{t_0}$: Dead time or retention time of the unretained compound (this is the time required for the mobile phase to pass through the column without interacting with the stationary phase).

- **Principle :**

- ✓ If **k′ = 0** , the compound is not retained and flows directly with the mobile phase.
- ✓ If **k′** is high, it means that the solute interacts strongly with the stationary phase and spends more time in it, thus increasing the retention time.
- ✓ In general, an optimal value of **k′** is between **1 and 10** to ensure good separation without excessively extending the analysis time.

- ***Example :***

Let's take a simple example to illustrate the calculation of the capacity factor:

Suppose that during a chromatographic analysis we have the following data:

- ***(solute retention time)** : 10 minutes*
- ***(time out)** : 2 minutes*

*The capacity factor **k'** = 4: This means that the solute spends 4 times more time in the stationary phase than in the mobile phase.*

- **Importance of Capacity Factor :**

- ✓ **Efficiency assessment** : Too low a **k'** indicates poor retention, which can lead to peak overlap. Too high a **k'** lengthens the analysis time unnecessarily.
- ✓ **Separation optimization** : The capacity factor helps to adjust the chromatographic conditions (type of stationary phase, choice of mobile phase) to obtain efficient separation of compounds.
- ✓ **Method comparison** : Using the capacity factor, different columns and mobile phases can be compared to assess which offers better retention and separation.

- **Relationship with other chromatographic parameters :**

The capacity factor is often used in conjunction with other parameters such as the **selectivity factor** (α) and the **number of theoretical plates** (N) to describe and optimize the separation process.

- **Selectivity factor (α)** : Defined by the ratio of the capacity factors of two compounds, it allows the resolution between two peaks to be evaluated.

$$\alpha = \frac{k'_2}{k'_1} \quad \left[k'_2 \triangleright k'_1 \right]$$

- **Application examples :**
- **High-performance liquid chromatography (HPLC)** : By adjusting the polarity of the mobile phase, the capacity factor of the analytes can be changed to improve separation.
- **Gas chromatography (GC)** : By changing the column temperature, the capacity factor of the analytes can be influenced.
- **Conclusion :**

The capacity factor is an essential tool in chromatography to evaluate and optimize separation conditions. By playing on experimental variables such as the nature of the stationary phase or the composition of the mobile phase, the capacity factor can be adjusted to obtain optimal separations and faster and more precise analyses.

(h) Selectivity factor (α) in chromatography:

- **Definition :**

The selectivity factor, denoted **α** , is a key parameter in chromatography that measures the ability of a chromatographic column to distinguish two different compounds. It indicates the difference in retention between two analytes on the same column and allows their separation t**o be evaluated.**

- **Formula :**

The selectivity factor is defined by the ratio of the capacity factors (k′) of two distinct analytes:

$$\alpha = \frac{k'_2}{k'_1}$$

- k'_2 : Capacity factor of the most retained compound ($k'_2 \triangleright k'_1$).
- k'_1 : Capacity factor of the least retained compound.

The selectivity factor is always greater than 1 ($\alpha > 1$

- **Principle :**

The selectivity factor is used to quantify the difference in retention between two solutes. The higher the selectivity factor, the greater the difference in retention, which results in a better separation of the two compounds.

- ✓ **($\alpha \approx 1$)** : The analytes are weakly separated or not separated at all (overlapping peaks).
- ✓ **(** $\alpha > 1$**)** : Indicates good separation of analytes.
- ✓ **Very high α** : Indicates a sharper separation, but if α is too large it may result in a longer analysis time for the most retained compound.

- ***Calculation Example :***

Suppose we have two compounds, A and B, analyzed by chromatography with the following parameters:

- *Retention time of compound A (t_{R1}): 5 minutes*
- *Retention time of compound B (t_{R2}): 10 minutes*
- *Timeout (t_0): 2 minutes*

Let's first calculate the capacity factors:

$$k_2' = \left(\frac{10-2}{2}\right) = 4 \quad \text{and} \quad k_1' = \left(\frac{5-2}{2}\right) = 1.5 \quad \text{So}: \alpha = 2.67$$

Interpretation: A selectivity factor of 2.67 means that compound B is significantly more retained than compound A, indicating good separation.

- **Importance of Selectivity Factor :**

- **Separation Optimization** : The selectivity factor is used to adjust experimental conditions to improve the separation of analytes.
- **Stationary Phase Selection** : Selectivity depends on the nature of the stationary phase. By changing the column, better separation of analytes can be achieved by increasing α .
- **Comparison of Analytes** : The selectivity factor allows to compare the retention of several compounds on the same column.

- **Improved Selectivity :**

- **Modification of the mobile phase** : By changing the polarity or composition of the mobile phase, one can influence the retention of analytes and increase the selectivity factor.
- **Changing the stationary phase** : Using a stationary phase with different chemical interactions can increase the retention difference between two analytes.
- **Temperature** : In gas chromatography (GC), adjusting temperature can affect retention times and improve selectivity.

- ***Practical Example :***

Let's assume an HPLC analysis where we want to separate two isomers: ibuprofen and naproxen.

- *Using a C18 column with an aqueous acetonitrile mobile phase (50:50), we observe that the retention times are very close, and α≈1.05.*

- *By adjusting the mobile phase composition to 60% acetonitrile, the retention time of naproxen increases more than that of ibuprofen, and α increases to 1.2.*
- *This improvement in selectivity (α) allows better separation of peaks on the chromatogram.*

- **Relationship with Resolution (Rs)** :

The selectivity factor is a key component of the resolution formula (Rs), which measures the separation between two chromatographic peaks:

$$R_S = \frac{\sqrt{N}}{4} \times \frac{\alpha - 1}{\alpha} \times \frac{k_2'}{1 + k_2'}$$

- **N** : Number of theoretical plates
- **α** : Selectivity factor
- $\mathbf{k_2'}$: Capacity factor of the most retained compound

- **Benefits** :
  - ✓ Allows to evaluate and optimize the separation of analytes.
  - ✓ Used to compare the efficiency of different columns and experimental conditions.
- **Limitations** :
  - ✓ An α close to 1 makes separation difficult and may require significant adjustments to chromatographic conditions.
  - ✓ If α is very high, the analysis time may be unnecessarily prolonged for some analytes.

Conclusion:

The selectivity factor (α) is an essential parameter to evaluate and optimize the separation of analytes in chromatography. It allows to understand and adjust the interactions between analytes and the stationary phase, directly influencing the quality of the separation and the resolution of chromatographic peaks. Adjusting the mobile phase, choosing the stationary phase and modifying experimental conditions are key methods to optimize selectivity and thus obtain accurate and reliable analyses.

VII.1.8 Mobile phase:

In chromatography, the eluent, or mobile phase, is the liquid or gas that flows through the chromatographic column and carries the components of the sample to be analyzed. Its main function is to carry these molecules along the stationary phase, which is fixed inside the column.

- ✓ **Interaction with stationary phase:** In the column, the sample is separated according to its interaction with the stationary phase and the mobile phase. Components that interact weakly with the stationary phase are carried away more quickly by the mobile phase and therefore have a shorter retention time, while those that interact more strongly remain in the column longer.

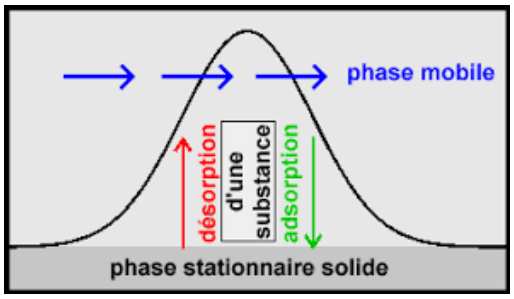

Fig. V.7: Interaction phase moble / stationnaire

- ✓ **Polarity of the eluent:** In liquid chromatography, for example, the polarity of the eluent is crucial, as it can influence the separation of components. For example, in normal-phase chromatography, a less polar eluent promotes the retention of polar compounds on the polar stationary phase. In contrast, in reversed-phase chromatography, a more polar mobile phase more easily carries away polar compounds.
- ✓ **Separation speed:** The speed of movement of the mobile phase also influences the resolution of the separation. A fast mobile phase reduces the analysis time, but may result in less separation of components (less well resolved peaks).

Thus, the mobile phase is essential for the movement of analytes in the column and directly affects the separation efficiency and the quality of analytical results.

VII.1.9 Stationary Phase:

VII.1.9.1 Definition:

The stationary phase is a key component in chromatography techniques. It is the solid or liquid material immobilized in a column or on a surface where the different compounds of the sample interact. Its role is to allow the separation of the different analytes according to their interactions with it, and with the mobile phase (the solvent or gas that passes through the stationary phase)

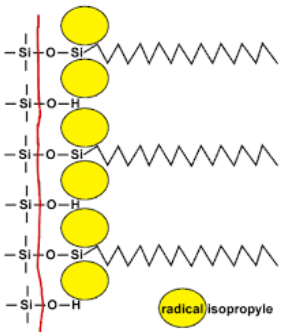

Fig. V.8: Stationary phase[1]

[1] https://www.analyticaltoxicology.com/chromatographie-liquide-haute-performance-hplc/

https://www.analyticaltoxicology.com/chromatographie-phase-gazeuse-cpg/

https://www.analyticaltoxicology.com/methodes-separatives/

VII.1.9.2 Principle:

The principle of the stationary phase is based on the differential interaction of analytes (molecules present in the sample) with the stationary phase. When a sample passes through the stationary phase with the help of the mobile phase, molecules that interact strongly with the stationary phase move more slowly, while those that interact less pass more quickly. This leads to a separation of the components according to their affinity with the stationary phase.

VII.1.9.3 Types of Stationary Phase:

There are several types of stationary phases used depending on the type of chromatography used:

- **Solid Stationary Phase (Adsorption Chromatography):**
  - ✓ Used mainly in adsorption chromatography, it consists of adsorbent solids such as silica gel, alumina, or activated carbon.
  - ✓ Analytes temporarily attach to the surface of stationary phase particles, causing them to separate.
- **Liquid Stationary Phase (Partition Chromatography):**
  - ✓ In partition chromatography, the stationary phase is a layer of liquid immobilized on a solid support.
  - ✓ Examples: Octadecylsilane (C18) used in high performance liquid chromatography (HPLC) is a common liquid stationary phase.
- **Polymer Stationary Phase:**
  - ✓ Used mainly in ion exchange chromatography.
  - ✓ Composed of charged polymer resins that interact with ions based on their opposite charges.
- **Gas Stationary Phase (Gas Chromatography - GC):**
  - ✓ The stationary phase is an immobilized liquid or polymer coating the interior of the capillary column.
  - ✓ The analytes dissolve in this liquid before redistributing into the mobile phase.

VII.1.9.4 Stationary Phase Forms:

- **Columns Filled:**
  - ✓ Packed columns contain stationary phase particles in powder or gel form.
  - ✓ Used in liquid chromatography.
- **Capillary Columns:**
  - ✓ In capillary columns (especially for GC), a thin layer of stationary phase is coated on the inner walls of a capillary tube.

VII.1.9.5 Interactions with the Stationary Phase:

Analyte-stationary phase interactions depend on several physical and chemical phenomena:

- **Adsorption:** Interaction of analytes with the surface of a solid stationary phase.

- ✓ Examples: Van der Waals interactions, London forces.

- **Partition:** Distribution of analytes between the liquid stationary phase and the mobile phase.
  - ✓ The molecules temporarily dissolve in the stationary phase before returning to the mobile phase.
- **Ion Exchange:** Charged analytes exchange their ions with ions of the opposite charged stationary phase.
  - ✓ Used for ionic compounds, amino acids, proteins.
- **Steric Exclusion:** The separation is done according to the size of the molecules.
  - ✓ Small molecules enter the pores of the stationary phase and are retained longer.

VII.1.9.6 Laws Governing Interactions:

- **Nernst's Law (Partition):**
  - ✓ Describes the distribution of an analyte between two phases (stationary and mobile).
- **Adsorption isotherms (Langmuir, Freundlich):**
  - ✓ Describe how an analyte adsorbs to the surface of a solid stationary phase.

VII.1.9.7 Choice of Stationary Phase:

- The choice of stationary phase depends on several factors:
- **Nature of Analytes:** Polarity, size, charge of analytes.
  - ✓ Examples:
    - Polar analytes often use polar stationary phases such as silica.
    - Non-polar analytes use non-polar phases such as C18.
- **Type of Chromatography:**
  - ✓ For adsorption chromatography, solids such as silica are common.
  - ✓ For reversed phase chromatography (HPLC), phases like C8 or C18 are chosen.
- **Compatibility with Mobile Phase:**
  - ✓ The stationary phase must be stable and insoluble in the mobile phase to avoid contamination and deterioration.

VII.1.9.8 Distribution of the Stationary Phase in the Column:

- **On the Surface:**
  - In liquid chromatography, the liquid stationary phase is immobilized on a solid surface such as silica beads.
- **Inside the Pores:**
  - For size exclusion chromatography, the stationary phase has pores into which small molecules can enter.

VII. 1 10 Sorption isotherms:

Sorption isotherms are mathematical models that describe how a solute (or analyte) interacts with an adsorbent surface (the stationary phase) at a constant temperature. These isotherms show the relationship between the amount of solute adsorbed per unit mass of adsorbent and the concentration of the solute in the mobile phase.

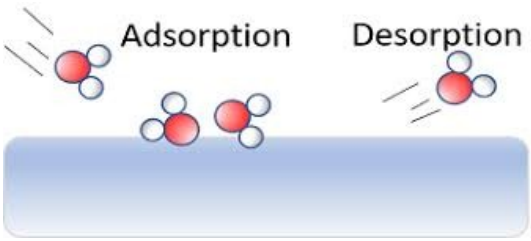

Fig. VII.9: Sorption phenomenon[2]

Sorption isotherms are particularly important in chromatography and materials analysis because they help to understand adsorption behavior and optimize separation processes**.**

VII. 1 10 .1 **Types of Sorption Isotherms:**

There are several types of adsorption isotherms that are commonly used to model the interaction between the adsorbent and the adsorbate:

(a) Langmuir **isotherm :**

❖ **Principle:**

- ✓ The Langmuir isotherm assumes that adsorption occurs on a homogeneous surface with a finite number of adsorption sites, where each site is equivalent.
- ✓ Once a molecule is adsorbed to a site, there is no further interaction with neighboring molecules, and each site can accommodate only one molecule.

• **Equation:**

$$q_e = \frac{K_L.C_e.q_{max}}{1+K_L.C_e}$$

- ✓ q_e : Quantity adsorbed at equilibrium (mg/g).
- ✓ q_{max} : Maximum adsorption capacity (mg/g).
- ✓ K_L : Langmuir constant (L/mg).
- ✓ C_e : Concentration of solute in mobile phase at equilibrium (mg/L).

• **Applications:**

[2] https://www.analyticaltoxicology.com/chromatographie-liquide-haute-performance-hplc/

https://www.analyticaltoxicology.com/chromatographie-phase-gazeuse-cpg/

https://www.analyticaltoxicology.com/methodes-separatives/

- ✓ Used for systems where adsorption reaches saturation, for example in adsorption chromatography for the purification of organic compounds.

(b) Freundlich isotherm:

- **Principle:**
  - ✓ The Freundlich isotherm is an empirical model that assumes a heterogeneous surface with adsorption sites of different energies. This model allows for multilayer adsorption.
  - ✓ It is often used to describe systems where adsorption is nonlinear and applies to both high and low concentrations.
- **Equation:**

$$q_e = K_F . C_e^{1/n}$$

- K_F : Adsorption capacity (mg/g)(L/mg).
- $1/n$: Heterogeneity index (unitless). $(1/n) < 1$, adsorption is favorable.

- **Applications:**
  - ✓ Used for heterogeneous systems, for example in the separation of complex aromatic compounds.

(c) BET (Brunauer, Emmett, Teller) isotherm:

- **Principle:**
  - ✓ The BET isotherm extends the Langmuir model for multilayer adsorptions. It considers that the molecules adsorbed on the surface form several layers and that the adsorption in the successive layers follows a geometric distribution.
- **Equation:**

$$q_e = \frac{q_{max} . C_e . K_{BET}}{(1 - C_e)[1 + (K_{BET} - 1) . C_e]}$$

K_{BET} : BET constant.

- **Applications:**
  - ✓ Used in the analysis of surfaces of solids, for example to determine the specific surface area of catalysts.

(d) Temkin isotherm:

- **Principle:**
  - ✓ The Temkin isotherm takes into account the interactions between the adsorbent and the adsorbate. It assumes that the heat of adsorption decreases linearly due to the interactions.
- **Equation:**

$$q_e = \frac{RT}{b} \ln(K_T C_e)$$

- ✓ b : Temkin constant.

- ✓ K_T : Adsorption equilibrium constant.

- **Applications:**
  - ✓ Used for systems where molecular interactions are significant, for example in the separation of volatile chemicals.

VII. 1 10.2 Practical Applications of Sorption Isotherms in Chromatography:

Adsorption isotherms help to understand how analytes distribute between the mobile phase and the stationary phase in chromatography. This helps to predict retention, elution, and separation efficiency.

- **Optimization of chromatographic conditions:** Knowing the isotherm, it is possible to adjust the mobile phase to obtain optimal separation.
- **Stationary phase capacity analysis:** Isotherms provide information on the maximum adsorption capacity of the stationary phase.
- **Chromatographic column design:** Isotherms allow selection of the type and amount of stationary phase for efficient separation.

- **Practical Example:**

Suppose we want to separate a mixture of two compounds A and B using liquid chromatography on a column packed with silica gel (solid adsorbent). The Langmuir isotherm can be used to model the retention of the compounds. If compound A has a stronger interaction with silica, its retention factor will be higher and it will elute more slowly than compound B. By adjusting the polarity of the mobile phase, we can modulate these interactions to achieve an efficient separation.

- **Conclusion :**

Sorption isotherms are essential tools for understanding and predicting interactions between analytes and the stationary phase in chromatography. Langmuir, Freundlich, BET, and Temkin models provide mathematical frameworks to describe these interactions and thus optimize the analytical separation process.

VII.1.11 Intermolecular interaction:

In chromatography, intermolecular interactions play a key role in the separation process. These interactions manifest themselves in different forms, such as electrostatic forces and Van der Waals forces. Here is a more detailed explanation of the main intermolecular forces and their role in chromatography.

(a) Electrostatic Forces:

- Definition: Electrostatic forces are forces of attraction or repulsion between two charged particles. They result from the interaction between positive and negative charges.
- Principle:
  - ✓ In chromatography, electrostatic forces are manifested mainly in ion exchange stationary phases.
  - ✓ Ions present in the mobile phase interact with charged sites in the stationary phase.
  - ✓ The stronger the ionic interaction, the higher the retention of the analyte.

(b) Van der Waals forces:

Van der Waals forces are a group of several types of weak but cumulative intermolecular interactions between neutral molecules. They fall into three main categories: **London dispersion forces** , **Debye induction forces** , and **Keesom orientation forces** .

b. 1. London Dispersion Forces:

Definition: London forces are attractive forces that arise between nonpolar molecules due to the formation of instantaneous and temporary dipoles.

Principle:

- These forces are due to a temporary fluctuation in the distribution of electrons in a molecule, creating an instantaneous dipole.
- They increase with the size and polarization of the molecules..

b.2 . Debye Induction Forces:

Definition : Debye forces (or induction forces) are forces of attraction between a polar molecule (having a permanent dipole) and a non-polar molecule.

Principle:

- The polar molecule induces a temporary dipole in the apolar molecule by attraction of electrons.
- These forces are influenced by the polarity of the polar molecule and the polarization capacity of the nonpolar molecule.

b.3 Keesom Orientation Forces:

Definition: Keesom forces (or dipole-dipole interactions) are attractive forces between two polar molecules having permanent dipoles.

Principle:

- The permanent dipoles of two molecules align in such a way as to minimize energy, creating an attraction between the opposite poles.
- These forces are temperature dependent: they decrease as the temperature increases.

(c) Hydrogen Bonds:

Definition: Hydrogen bonding is a strong interaction between a hydrogen atom bonded to an electronegative atom (such as O, N, or F) and another electronegative atom possessing a lone pair of electrons.

Principle:

- Hydrogen bonding strongly influences the retention of analytes in chromatography.
- The more hydrogen bonding opportunities there are, the more analyte is retained in the column.

(d)Hydrophobic Interactions:

Definition: Hydrophobic interactions occur when nonpolar molecules group together to avoid contact with an aqueous phase.

Principle:

- These interactions play an important role in reversed-phase chromatography (HPLC), where nonpolar analytes are retained by the hydrophobic stationary phase (such as C18).

- The more apolar the analyte, the stronger the hydrophobic interaction and the higher the retention.

❖ **Choice of Stationary Phase:** The choice of stationary phase depends on the intermolecular interactions desired for the separation:

- **Nonpolar phases** (C18, C8) for nonpolar analytes (hydrocarbons).
- **Polar phases** (silica, cyano) for polar analytes (alcohols, amines).
- **Ion exchange phases** for ionized analytes (amino acids, proteins).

❖ **Distribution in the Column: The distribution of analytes between the mobile phase and the stationary phase depends on:**

- **The affinity** of the analyte for the stationary phase (based on intermolecular interactions).
- **The flow rate** of the mobile phase.
- **The type of stationary phase** used (film-covered surface, solid particles).

Conclusion:

Intermolecular interactions are critical to separation mechanisms in chromatography. Understanding these forces allows for the selection of the appropriate stationary phase and optimization of mobile phase conditions for efficient separation of analytes, while improving resolution and efficiency of the analysis.

This includes an in-depth understanding of London, Debye and Keesom forces, as well as hydrophobic interactions and hydrogen bonding, which influence the retention and selectivity of analytes in a chromatographic analysis.