1. Mathematical models of the chromatographic process

- What determines retention time in LC?
- What causes peak broadening in LC?
- Why are the LC peaks often asymmetric?
- Why is partition chromatography much more popular than desorption chromatography?

Mathematical modeling of chromatography

- We will look at three aspects:
  - Compound’s retention time
  - Peak width
  - Peak shape

Partition and adsorption chromatography

- Partition chromatography:
  - Liquid-liquid chromatography
  - The most common is reversed phase (RP) LC
  - Gas-liquid chromatography
  - Partition between liquid and gas
- Adsorption chromatography:
  - Liquid-solid chromatography
  - Adsorption on a solid from a liquid
  - Gas-solid chromatography
  - Adsorption on a solid from the gas phase

Partition between liquid and gas

- Liquid-gas partition: **Henry’s law**
  \[ C_i = k_{H,i} \cdot P_i \]
  - \( C_i \) molar concentration of compound i in the liquid
  - \( P_i \) Partial pressure of compound i in the gas phase
  - \( k_{H,i} \) Henry’s constant of compound i
  - Depends on compound, liquid, temperature

Partition between two liquids

- Liquid-liquid: **Distribution law**
  \[ K_{d,i} = \frac{C_i^{v2}}{C_i^{v1}} \]
  \[ C_i^{v2} = K_{d,i} \cdot C_i^{v1} \]
  - \( C_i^{v1} \) molar concentration of compound i in liquid 1
  - \( C_i^{v2} \) molar concentration of compound i in liquid 2
  - \( K_{d,i} \) Distribution coefficient of i between liquids 1 and 2
  - \( K_{d,i} \) depends on compound and liquids, less on temperature
Linear relationship

- Both relationships give the same linear relationship – linear sorption isotherm (Henry isotherm):

\[ C_{\text{stats}} \]

\[ C_{\text{mob}} \]

\[ K_{d,i} \]

Linear chromatography

- Chromatographic model whereby linear sorption isotherm describes partition of the analyte molecules between stationary and mobile phases is called linear chromatography.

- If possible then it is useful to work under the linear chromatography conditions:
  - This is a lot easier to achieve in partition chromatography than in adsorption chromatography.

When does the linear chromatography model hold?

- Both stationary phase (SP) and mobile phase (MP) are homogenous
  - There is no possibility that the molecules of the same compound \( i \) can be retained with different strengths in either phase
- The volumes of the stationary and mobile phases are much larger than the amount of compound \( i \)
  - There is no interaction between the molecules of compound \( i \)
- In reality these conditions only partially hold

Distribution law in LC

\[ K_{d,i} = \frac{C_{\text{stats}}}{C_{\text{mob}}} \]

\( n_{\text{stats}} \)

\( n_{\text{mob}} \)

\( V_{\text{stats}} \)

\( V_{\text{mob}} \)

- \( n_{\text{stats}} \): number of moles of \( i \) in SP
- \( n_{\text{mob}} \): number of moles of \( i \) in MP
- \( V_{\text{stats}} \): volume of SP
- \( V_{\text{mob}} \): volume of MP

Capacity factor

\[ K_{d,i} \cdot V_{\text{stats}} = n_{\text{stats}} / n_{\text{mob}} = k'_{i} \]

\( k'_{i} \)

- \( k'_{i} \): capacity factor of \( i \)
  - Depends on \( i \), \( K_{d,i} \), and volumes of the phases
Capacity factor

- Since:
  \[ k_i' = \frac{t_{R,i}}{t_M} \]
- then:
  \[ K_{d,i} \cdot \frac{V_{\text{stats}}}{V_{\text{mob}}} = \frac{t_{R,i} - t_M}{t_M} \]
- \( t_{R,i} \) retention time of i
- \( t_M \) dead time of the system

Retention time of compound i

- After rearranging:
  \[ t_{R,i} = t_M \cdot \left( \frac{K_{d,i} \cdot V_{\text{stats}}}{V_{\text{mob}}} + 1 \right) \]
- This is the Main equation of elution
- Compound i is retained the stronger,

Peak width

- Peak width increases on elution
  - i.e. separation efficiency decreases
- This is described by the van Deemter equation

van Deemter equation

\[ H = A + B u + C_s u + C_M u \]

- \( H \) – height of the theoretical plate (HETP)
- \( u \) – linear flow rate of MP
- \( A \) – Eddy diffusion term (several flow paths)
- \( B \) – longitudinal diffusion term
- \( C_s, C_M \) - mass transfer coefficients in SP and MP

How will efficiency change if we ...

- Make particles more uniform?
- Make particles smaller?
- Increase column temperature?
- Increase the viscosity of the mobile phase?
Peak shape

- If
  - The sorption isotherm is linear (i.e. Henry isotherm)
  - There are many factors causing peak broadening
  - The factors act in both directions
  - The factors influence all analyte molecules with the same probability, i.e. without differentiating molecules in different parts of the peak

- Then the peak has the shape of the Gauss distribution (Normal distribution)

In real life peaks usually have tails

Asymmetry factor

Optimal values of the parameters

- Tailing is a problem:
  - Resolution deteriorates
  - Quantitative accuracy becomes worse
  - Limit of detection gets higher

- An acceptable value: $A_s < 2$

- Why are peaks often asymmetric?

Nonlinear sorption isotherm

- Also in partition LC the sorption isotherm is often nonlinear
- The most common reason in reversed phase LC:
  - Dual retention: partition + adsorption
- Adsorption occurs on
  - Residual silanol groups
  - Metal cations, present in silica as impurities
RP stationary phases

- The majority of stationary phases are based on silica
- On silica surface: **Silanol groups**
- These are derivatized during production:

\[
\text{Si-OH} + \text{Cl-SiR}_3 \rightarrow \text{Si-O-SiR}_3 + \text{HCl}
\]
- The fuller is the derivatization, the better
- Some always remain underivatized: **residual silanol groups**

How to minimize the number of residual silanols?

- **End-capping**
  - Besides Cl-Si(CH₃)₂-R some smaller-molecule derivatization reagent is added
- **Shielding**
  - Instead of Cl-Si(CH₃)₂-R the Cl-Si(t-Bu)₂-R reagent is used

Silanol groups

- Silanol groups are of three types (simplified):

  a) isolated  
  b) geminal  
  c) vicinal

- The adsorption ability is the stronger, the higher is the **acidity**
  - The \( pK_a \) values range from 3 to 15

Metal cations

- Metal cations greatly enhance the adsorption ability of silica
- They can be
  - Free (d) or
  - Embedded in the lattice (e)
- Because of their valence properties the metal cations are positively charged in the lattice

\[
\text{M} + \text{Si-OH} \rightarrow \text{M-Si} \quad \text{d)} \\
\text{M} \quad \text{e)}
\]

Adsorption depends on the compound

- Such adsorption influences first of all:
  - Polar compounds
  - Especially strongly: basic compounds
  - Often a base is added to the MP
  - Compounds that give strong metal complexes

Physical background?

- Two sorption processes run in parallel:
  - **1. Partition**
    - Large volumes of phases
    - All analyte molecules “have space” in the SP
    - Linear isotherm
  - **2. Adsorption**
    - Small number of adsorption centres
    - Only a small part of the analyte molecules can be adsorbed
    - Retention by adsorption is stronger than by partition
Modeling adsorption

• Assumptions (1):
  – Adsorption only occurs on adsorption centres
  • Monomolecular adsorption
  – Analyte molecules do not interact with each other
  – The number of centres is limited
  – All centres are energetically equivalent

Modeling adsorption

• Assumptions (2):
  – There are two processes running simultaneously: adsorption and desorption
  – Their relative rates determine the adsorption equilibrium
  – The rate of adsorption is proportional to the number of free centres and the number of non-adsorbed analyte molecules in the liquid phase
  – The rate of desorption is proportional to the number of occupied centres

Adsorption rate

\[ v_{ads} = k_{ads} \cdot C_{i}^{mob} \cdot (C_{i}^{ads\_max} - C_{i}^{ads}) \]

• Adsorption rate
• Rate constant of adsorption
• Concentration of analyte in the MP
• Maximum surface concentration of analyte molecules
• Surface concentration of analyte molecules
• The extent of surface occupation: \( \theta = \frac{C_{i}^{ads}}{C_{i}^{ads\_max}} \)

Desorption rate

\[ v_{des} = k_{des} \cdot C_{i}^{ads} \]

• Desorption rate
• Desorption rate constant
• Surface concentration of analyte molecules

Equilibrium

• There is equilibrium if
  \[ v_{ads} = v_{des} \]

• Therefore:
  \[ k_{ads} \cdot C_{i}^{mob} \cdot (C_{i}^{ads\_max} - C_{i}^{ads}) = k_{des} \cdot C_{i}^{ads} \]

Analyte concentration on surface

• Reorganising:
  \[ C_{i}^{ads} = C_{i}^{ads\_max} \frac{k_{ads}}{k_{ads} + k_{des}} C_{i}^{mob} \]

• Bringing in adsorption equilibrium constant:
  \[ K_{i}^{ads} = \frac{k_{ads}}{k_{des}} \]
Langmuir’s isotherm

- We get the **Langmuir’s** isotherm:

\[ C_{i_{\text{ads}}} = C_{i_{\text{ads max}}} \frac{K_{i_{\text{ads}}}}{1 + \frac{C_{i_{\text{mob}}}}{K_{i_{\text{ads}}}}} \]

Shape of Langmuir’s isotherm

- If the number of centres is large compared to the number of analyte molecules then the isotherm can be approximated by the **Henry isotherm**
- If the number of centres is small then almost all of them are occupied

Drawbacks of Langmuir’s isotherm

- Adsorption centres are not energetically equivalent
- Analyte molecules interact among themselves.
- Adsorption can occur on molecules already adsorbed. So the assumption of monomolecularity does not hold

The overall sorption isotherm

- Mixed **nonlinear** isotherm

Peak shape with nonlinear isotherm

- Gaussian peak
  - 100 adsorption-desorption processes
  - 500 adsorption-desorption processes

Detektori signaal

Gaussi kõvera kujuline piik

100 adsorptsiooni-desorptsiooniprotsessi

500 adsorptsiooni-desorptsiooniprotsessi

Retentsiooniaeg, min
Conclusions (1)

• It is good if the chromatographic process is based on partition, with linear sorption isotherm
  – Symmetric peaks
• For this: either no adsorption centres or they must be occupied

Stationary phase must be of high quality:
- low number of residual silanols and metal cations
- their activity low:
  - endcapping, shielding
  - acid washing

Mobile phase additive can be used, which adsorbs strongly and does not let analyte molecules to adsorb

Conclusions (2)

• In adsorption chromatography the work should be done in the “Henry region”:
  – Large specific surface
  – Lots of centres on the surface
  – Centres energetically similar
  – Low amounts of analytes should be injected
• In reality it is almost never ideal
  – Tailing is frequent in adsorption chromatography

• This is one of the reasons why partition chromatography is more popular than adsorption chromatography

Conclusions (3)

• Tailing is more pronounced with analytes that adsorb stronger
  – First of all polar and basic compounds
  – Also compounds that give strong metal complexes

Many other adsorption models

• Freundlich’s isotherm
  – Monomolecular adsorption
  – Centres are not energetically equivalent
  – The activity of the centres decreases logarithmically

• Tjomkin’s isotherm
  – Analogous, but the decrease is linear

• BET isotherm
  – Polymolecular adsorption

Many more empirical models

• Ca 90 empirical models have been proposed for describing peak shape
  – All describe unsymmetrical peaks
  – Some even describe doubled peaks

Applications of the models

• Deeper understanding
• Peak deconvolution
• LC simulations