

# 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 adsorption chromatography?

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## Mathematical modeling of chromatography

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

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## Partition and adsorption chromatography

- **Partition** chromatography:
  - Liquid-liquid chromatography
    - **Partition** between two liquids
    - The most common is **reversed phase** (RP) LC
  - Gas-liquid chromatography
    - **Partition** between liquid and gas
- **Partition** is sometimes also called **distribution**

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## Partition and adsorption chromatography

- **Adsorption** chromatography:
  - Liquid-solid chromatography
    - **Adsorption** on a solid from a liquid
  - Gas-solid chromatography
    - **Adsorption** on a solid from the gas phase

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## 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*

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## Partition between two liquids

- Liquid-liquid: **Distribution law**

$$K_{d,i} = \frac{C_i^{v2}}{C_i^{v1}} \quad C_i^{v2} = K_{d,i} \cdot C_i^{v1}$$

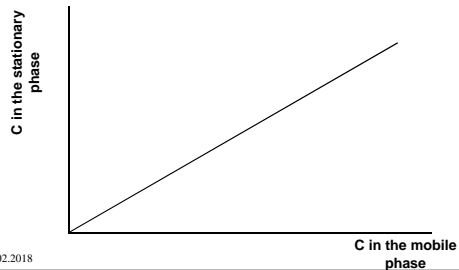
- $C_i^{v1}$  molar concentration of compound i in liquid 1
- $C_i^{v2}$  molar concentration of compound i in liquid 2
- $K_{d,i}$  Distribution coefficient of i between liquids 1 and 2
  - $K_{d,i}$  depends on compound and liquids, less on temperature

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## Linear relationships

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



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## 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

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## 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

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## Distribution law in LC

$$K_{d,i} = \frac{C_i^{\text{stats}}}{C_i^{\text{mob}}}$$

$C_i^{\text{stats}}$

concentration of *i* in SP

$C_i^{\text{mob}}$

concentration of *i* in MP

$K_{d,i}$

Distribution coefficient of *i* between SM and MP

–  $K_{d,i}$  depends on the compound and the phases, less on temperature

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## Distribution law in LC

- After rearranging:

$$K_{d,i} = \frac{\frac{n_i^{\text{stats}}}{V^{\text{stats}}}}{\frac{n_i^{\text{mob}}}{V^{\text{mob}}}}$$

- $n_i^{\text{stats}}$  number of moles of *i* in SP
- $n_i^{\text{mob}}$  number of moles of *i* in MP
- $V^{\text{stats}}$  volume of SP
- $V^{\text{mob}}$  volume of MP

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## Capacity factor

- After rearranging:

$$K_{d,i} \cdot \frac{V^{\text{stats}}}{V^{\text{mob}}} = \frac{n_i^{\text{stats}}}{n_i^{\text{mob}}} = k'_i$$

- $k'_i$  capacity factor of *i*
  - Depends on *i*,  $K_{d,i}$ , and volumes of the phases

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## Capacity factor

- Since:

$$k'_i = \frac{t_{R,i} - t_M}{t_M}$$

- then:

$$K_{d,i} \cdot \frac{V^{stats}}{V^{mob}} = \frac{t_{R,i} - t_M}{t_M}$$

- $t_{R,i}$  retention time of i
- $t_M$  dead time of the system

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## Retention time of compound i

- After rearranging:

$$t_{R,i} = t_M \cdot \left( K_{d,i} \cdot \frac{V^{stats}}{V^{mob}} + 1 \right)$$

- This is the **Main equation of elution**
- Compound i is retained the stronger,

What factors cause strong retention?

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## Peak width

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

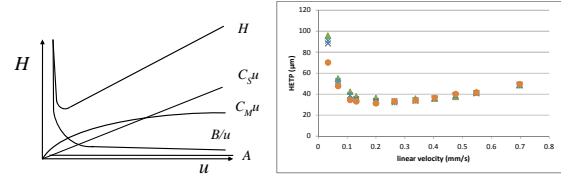
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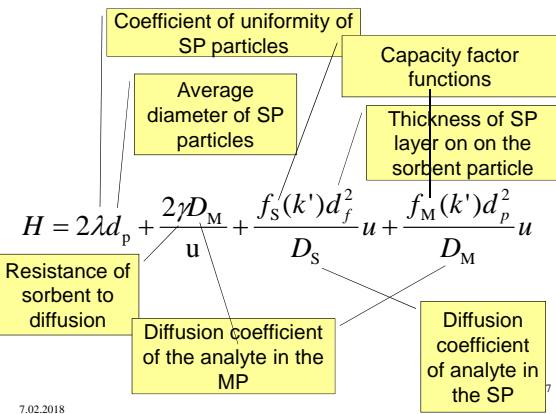
## van Deemter equation

$$H = A + B/u + C_S u + C_M u$$

- $H$  – 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



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## How will efficiency change if we ...

... Make particles more uniform?

... Make particles smaller?

... Increase column temperature?

... Increase the viscosity of the mobile phase?

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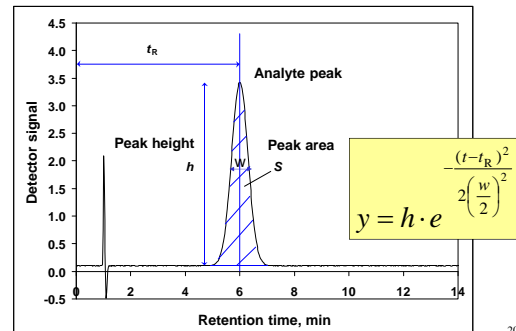
## Peak shape

- 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)**

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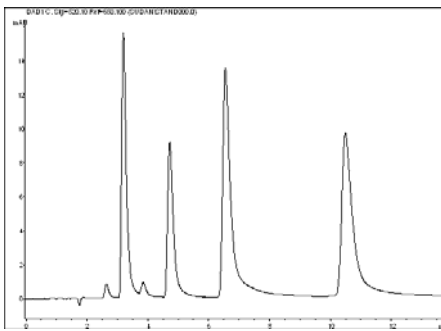
## Peak with Gauss shape



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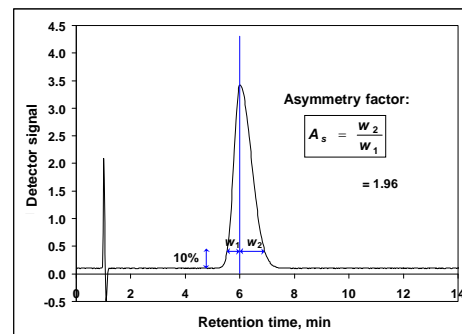
## In real life peaks usually have tails



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## Asymmetry factor



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## 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?**

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## Nonlinear sorption isotherm

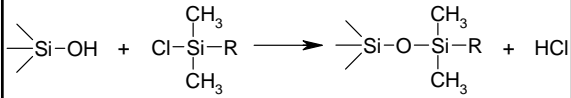
- Also in partition LC the sorption isotherm is often nonlinear
- The most common reason in reversed phase LC:
  - Dual retention: partition + **adsorption**
- Adsorption occurs on
  - Residual silanol groups
  - Metal cations, present in silica as impurities

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## RP stationary phases

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



- The fuller is the derivatization, the better
- Some always remain underivatized: **residual silanol groups**

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## How to minimize the number of residual silanols?

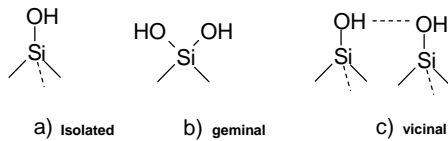
- *End-capping*
  - Besides Cl-Si(CH<sub>3</sub>)<sub>2</sub>-R some smaller-molecule derivatization reagent is added
- *Shielding*
  - Instead of Cl-Si(CH<sub>3</sub>)<sub>2</sub>-R the Cl-Si(t-Bu)<sub>2</sub>-R reagent is used

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## Silanol groups

- Silanol groups are of three types (simplified):



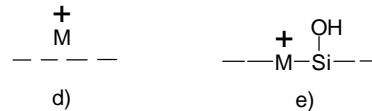
- The adsorption ability is the stronger, the higher is the **acidity**
  - The pK<sub>a</sub> values range from 3 to 15
    - Usually 5 .. 8

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## 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



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## 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

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## 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

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## 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**

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## 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

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## Adsorption rate

$$v_{\text{ads}} = k_i^{\text{ads}} \cdot C_i^{\text{mob}} \cdot (C_i^{\text{ads\_max}} - C_i^{\text{ads}})$$

- 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:  $\theta = \frac{C_i^{\text{ads}}}{C_i^{\text{ads\_max}}}$

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## Desorption rate

$$v_{\text{des}} = k_i^{\text{des}} \cdot C_i^{\text{ads}}$$

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

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## Equilibrium

- There is equilibrium if

$$v_{\text{ads}} = v_{\text{des}}$$

- Therefore:

$$k_i^{\text{ads}} \cdot C_i^{\text{mob}} \cdot (C_i^{\text{ads\_max}} - C_i^{\text{ads}}) - k_i^{\text{des}} \cdot C_i^{\text{ads}} = 0$$

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## Analyte concentration on surface

- Reorganising:

$$C_i^{\text{ads}} = C_i^{\text{ads\_max}} \frac{k_i^{\text{ads}} \cdot C_i^{\text{mob}}}{k_i^{\text{ads}} \cdot C_i^{\text{mob}} + k_i^{\text{des}}}$$

- Bringing in adsorption equilibrium constant:

$$K_i^{\text{ads}} = \frac{k_i^{\text{ads}}}{k_i^{\text{des}}}$$

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## Langmuir's isotherm

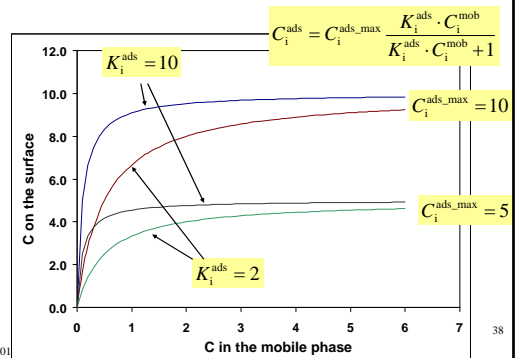
- ... We get the **Langmuir's** isotherm:

$$C_i^{\text{ads}} = C_i^{\text{ads\_max}} \frac{K_i^{\text{ads}} \cdot C_i^{\text{mob}}}{K_i^{\text{ads}} \cdot C_i^{\text{mob}} + 1}$$

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## Shape of Langmuir's isotherm



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## 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**

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## Drawbacks of Langmuir's isotherm

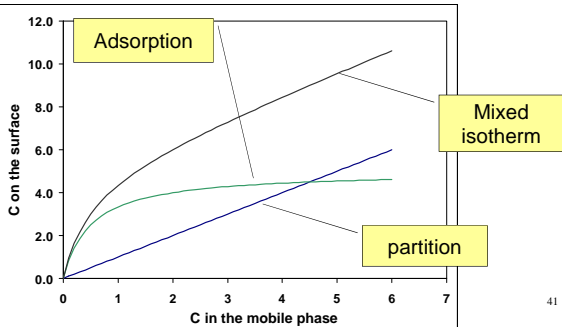
- Adsorption centres are not energetically equivalent
- Analyte molecules interact among themselves.
- Adsorption can occur on molecules already adsorbed. So the assumption of monomolecularity does not hold

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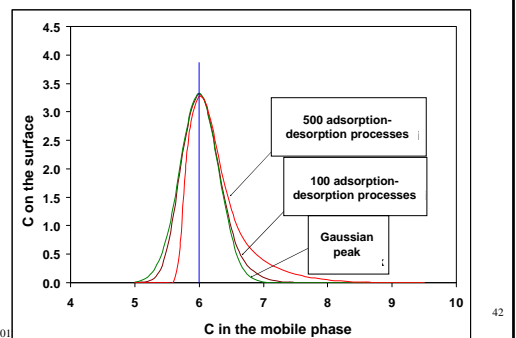
## The overall sorption isotherm

- Mixed **nonlinear** isotherm



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## Peak shape with nonlinear isotherm



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### 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**

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

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

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### 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

Why?

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### 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

Why?

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### Many other adsorption models

- **Freundlich's isotherm**
  - Monomolecular adsorption
  - Centres are not energetically equivalent
  - The activity of the centres decreases logarithmically
- **Tjorkin's isotherm**
  - Analogous, but the decrease is linear
- **BET isotherm**
  - Polymolecular adsorption

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### Many more empirical models

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

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V.B. Di Marco, G.G. Bombi *J. Chrom. A* 2001, 931, 1-30

### Applications of the models

- Deeper understanding
- Peak deconvolution
- LC simulations

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