Course „Modern Analytical Methods in Chemical Industry“

Chromatographic Techniques
Principle

**Gas chromatography (GC):**
- Mobile phase: 
  - Gases: \( H_2, N_2, Ar, He \) 
- Stationary phase: 
  - Capillary or packed columns with substituted siloxanes 
- Detectors: 
  - TCD, FID, MSD 
- Applicability: 
  - Volatile organic compounds, permanent gases

**High pressure liquid chromatography (HPLC):**
- Mobile phase: 
  - \( H_2O, \) organic solvents 
- Stationary phase: 
  - Impregnated plates 
- Detectors: 
  - UV-VIS, RI, MSD 
- Applicability: 
  - Organic compounds and inorganic salts

**Thin layer chromatography (TLC):**
- Mobile phase: 
  - Vapor of organic solvents 
- Stationary phase: 
  - Impregnated plates 
- Detectors: 
  - Optical detection (UV-VIS, fluorescence) 
- Applicability: 
  - Organic compounds

---

*large number of standardized procedures (norms) for industrial and environmental analysis*
Fundamentals of Chromatography

Sample injection \((t = 0)\)

- \(t_0\) - "death time" = retention of a compound with no interaction with the stationary phase
- \(t_{R(1)}\) - total retention time of component 1
- \(t_{R(2)}\) - total retention time of component 2
- \(t'_{R(1)}\) - net retention time of component 1 \((= t_{R(1)} - t_0)\)
- \(t'_{R(2)}\) - net retention time of component 2 \((= t_{R(2)} - t_0)\)
- \(\omega_{(1)}\) - base peak with of component 1
- \(\omega_{(2)}\) - base peak with of component 2
Equations in Chromatography (1)

Capacity factor $k'$
(ratio between the residence times of the sample in the stationary and the mobile phase)

$$k' = \frac{t'_R}{t_0} = \frac{t_R - t_0}{t_0} = \frac{t_R}{t_0} - 1$$

Relative retention/Separation factor/Selectivity $\alpha$

$$\alpha = \frac{k'_{(2)}}{k'_{(1)}}$$

→ Separation of two components is only possible if $\alpha > 1$

Linear velocity $u$

$$u = \frac{L}{t_0} = \frac{F}{q}$$

L - length of the column
$t_0$ - dead time
F - Feed ratio of the mobile phase [ml/s]
q - free cross-sectional area of the column
Equations in Chromatography (2)

Porosity of a column $\varepsilon_T$

$$\varepsilon_T = \frac{q}{\pi \cdot r^2} = \frac{F \cdot t_0}{\pi \cdot r^2 \cdot L} = \frac{F \cdot t_0}{V_R}$$

- $r$ - radius of the column
- $V_R$ - volume of the empty column

Permeability $K$

$$K = \frac{u \cdot L}{\Delta p} = \frac{L^2}{\Delta p \cdot t_0}$$

- $\Delta p$ - pressure difference between inlet and outlet of a column

large $K$ - wrong particle packing, low $K$ - clogging

Specific permeability $K^0$

$$K^0 = K \cdot \eta \cdot \varepsilon_T = \frac{d_p}{1000}$$

- $\eta$ - viscosity of the mobile phase
- $d_p$ - particle diameter of the packing
Equations in Chromatography (3)

Peak broadening

\[ N = \frac{16}{(t_{R(i)} \cdot \sigma_{(i)})^2} = \frac{5.54}{(t_{R(i)} \cdot \sigma_{0.5,(i)})^2} \]

- \( N \) - number of plates
- \( \sigma \) - base peak with
- \( \sigma_{0.5} \) - peak with at half peak height

\[ H = \frac{L}{N} \]

- \( H \) - height of plates

Effective number of plates \( N_{\text{eff.}} \) (independent on components of the sample)

\[ N_{\text{eff.}} = N \cdot \left( \frac{k'}{k' + 1} \right)^2 = 16 \cdot \left( \frac{t'_{R}}{\sigma} \right)^2 \]
Equations in Chromatography (4)

Resolution $R$ between 2 peaks

$$R = 2 \cdot \frac{t'_{R(2)} - t'_{R(1)}}{\bar{\omega}_{(1)} + \bar{\omega}_{(2)}} \approx \frac{\Delta t'}{\bar{\omega}}$$

(for peaks close one to the another)

$$R = \frac{1}{4} \cdot \frac{(\alpha - 1)}{\alpha} \cdot \frac{k'_{(2)}}{k'_{(2)} + 1} \cdot \sqrt{N}$$

- selectivity term
  (determined by stationary and mobile phases)

- capacity term
  (only significant for $0 < k'_{(2)} < 5$, for large $k'_{(2)} \rightarrow 1$)

- effectivity term
  (determined by $d_p$, $L$ and $u$)

$\rightarrow R = 1 \rightarrow 98\%$ peak separation, $R = 1.5 \rightarrow$ fully separated peaks
Equations in Chromatography (5)

Van Deemter equation

\[ H = A + \frac{B}{u} + C \cdot u \]

- Eddy diffusion term (peak broadening by different ways of sample molecules in a column)
- Axial diffusion term (statistic axial distribution of sample molecules)
- Term for mass exchange between stationary and mobile phases (adsorption/desorption, diffusion/back diffusion)
Gas Chromatography

Samples:  
- gaseous and liquid samples  
- limitations: compound which should be analyzed, should be stable under GC operation conditions and should have a vapor pressure significantly higher than zero  
- sample preparation: filtration, extraction, if necessary derivatisation (= conversion of „critical“ substances to such with higher stability and vapor pressure, e.g. carbon acids to esters)

Mobile phases:
- He, H₂, Ar, N₂ (purity 99.999 % or better)

Duration of an analysis:
- 5 ... 60 min (standard GC), < 2 min (Micro GC)

Application:  
- purity control, quality management and certification (wide application)  
- environmental and pharmaceutical analysis  
- analysis of main and trace components (% to ppm)
GC Setup

- Autosampler for liquid samples
- Injection vials of autosampler
- FID and TCD
- Status display for operation parameters
- Computer for GC operation and data handling
- MSD
- GC oven with column(s)
- Injector
- Keyboard for controlling flow rates and temperatures
Sample Injection

Liquid samples:
- manual (using a syringe, 0.1 – 10 µl, identification of compounds)
- automated by autosampler (need of 1 - 2 ml sample solution, high reproducibility, use for quantitative analysis)
- “on column” injection for samples with low thermal stability
  (injection directly on top of the cold column, than slow heating)
  → high precision, but danger of column overloading and pollution
- temperature programmed vaporization – PTV
  (injection into the cold injector, than temperature programmed heating and vaporization)
  → high sensitivity and reproducibility, protection of the column
- “head space” injection - sampling of the vapor phase over the sample
  (useful if the sample contains solid particles)

Gaseous samples
- manual (using a gas-dense syringe, 5 – 50 µl)
- automated by gas sampling valves (0.25 - 5 ml)

Injector types:
- split-splitless injector
- volatile interface
**GC Columns**

**Capillary columns**
- thin layer of stationary phase
- \( d = 0.1 - 0.53 \) mm, \( l = 10 - 50 \) m,
- film thickness: \( 0.3 - 50 \) µm

**Packed columns**
- small particles of stationary phase
- \( d = 0.53 \) mm, \( l = 0.5 - 10 \) cm,
- particle diameter: \( 45 - 120 \) mesh

Retention behavior depends on:
- polarity of sample and stationary phase
- volatility of the sample compound
- velocity of the carrier gas (mobile phase)
- temperature
  - → temperature programs

**Stationary phases**
- silicon polymers (polysiloxanes, Si-O-R)
  - \( R = \) methyl – non-polar,
  - \( R = \) phenyl or cyanopropyl – intermediate polarity,
  - \( R = \) ethylene glycol or fluorinated hydrocarbon – polar,
  - variation of polarity by co-polymerization
- PLOT phases (porous layer open tubular)
  - small particles immobilized in the wall,
  - for separation of high volatile compounds,
  - typical stationary phases:
    - \( \text{Al}_2\text{O}_3 \), molsieve 5A,
    - polystyrene-divinylbenzene (DVB)
- Selected column manufactures:
  - J & W (Agilent),
  - Chrompack, Restek, Supelco
## GC Detectors

<table>
<thead>
<tr>
<th>Name</th>
<th>Selectivity</th>
<th>Typical minimum detectable level</th>
<th>Linear dynamic range</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCD, Thermal conductivity detector</td>
<td>non-selective (anything what differs from the carrier gas)</td>
<td>400 pg/ml carrier</td>
<td>$10^6$</td>
</tr>
<tr>
<td>FID, Flame ionization detector</td>
<td>materials that are ionized in air/H₂ flames (e.g. hydrocarbons)</td>
<td>5 pg C/s</td>
<td>$10^7$</td>
</tr>
<tr>
<td>MSD, Mass selective detector</td>
<td>tunable for any species</td>
<td>10 ng (SCAN)</td>
<td>$10^5$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 pg (SIM)</td>
<td></td>
</tr>
<tr>
<td>ECD, Electron capture detector</td>
<td>halogens</td>
<td>0.1 pg Cl/s</td>
<td>$10^4$</td>
</tr>
<tr>
<td>NPD, Nitrogen phosphorus detector</td>
<td>N, P, heteroatoms</td>
<td>0.4 pg N/s</td>
<td>$10^4$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2 pg P/s</td>
<td></td>
</tr>
<tr>
<td>FTIRD, Fourier transformed infrared</td>
<td>molecular vibrations (e.g. organic compounds)</td>
<td>&gt; 1 ng (depending on absorption)</td>
<td>$10^3$</td>
</tr>
<tr>
<td>detector</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AED, Atom emission detector</td>
<td>tunable for any element</td>
<td>0.1 – 20 pg/s (depending on the element)</td>
<td>$10^4$</td>
</tr>
</tbody>
</table>
Flow diagram of a commercially available TCD cell. In the left diagram, the switching flow causes the column effluent to pass through the filament channel. When the switching flow changes (right diagram), the column effluent will pass through the empty channel. During this time the filament channel fills with the switching gas, and reference measurements are made. Switching between the column effluent and reference gas occurs every 100 milliseconds.
Flame Ionization Detector (FID)
Mass Selective Detector (MSD)

Ionization and Fragmentation of the sample molecules by the electron beam

Electron source

Sample beam from MSD interface (pressure reduction to vacuum)

Quadrupol mass filter

Detector (SCAN or SIM mode)

Ionization and fragmentation:
1. \( M + e^- \rightarrow M^+ + 2 \ e^- \) (yielding molar peak \( M^+ \))
2. \( M^+ \rightarrow Z^+ + (M - Z) \)
   \( Z^+ \rightarrow Y^+ + (Z - Y) \)
   .... (yielding characteristic mass peaks and mass differences)
3. Rearrangement reactions

\( \rightarrow \) characteristic mass spectra, identification of substances possible
GC Application Examples

Pharmaceutical analysis:
Alkaloid street drugs

Environmental analysis:
Chlorinated pesticides

Petrochemical analysis:
Refinery gas
High Pressure Liquid Chromatography (HPLC)

- high pressure pumps
- 6 port injection valve
- column with stationary phase (normally at room temperature)
- detector

0.01 – 10 ml/min at pressures up to 500 bars

Sample (mixture)

Samples:
- liquid samples
- limitations: solubility in the mobile phase, no thermal restrictions
- sample preparation: filtration, extraction

Duration of an analysis:
- 5 ... 60 min

Application:
- purity control, quality management and certification (wide application)
- environmental and pharmaceutical analysis
- analysis of main and trace components (% to ppm)

<table>
<thead>
<tr>
<th>Mobile Phase</th>
<th>Stationary Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Phase HPLC</td>
<td>non-polar (e.g. hydrocarbons)</td>
</tr>
<tr>
<td>Reversed Phase HPLC</td>
<td>polar (H₂O, buffer solutions, alcohols, acetonitrile and mixtures of them)</td>
</tr>
</tbody>
</table>
HPLC Setup

Storage for mobile phase (eluent)

Filtration and degassing
Preparation of eluent mixtures

Pump

Autosampler

Column box

Injection valve

Control unit

Operation modes:
- isocratic: constant eluent composition
- gradient: continuous variation of eluent mixture during analysis

Detector unit with UV-VIS lamp
HPLCInjectors

6 port injection valve
(similar to gas sampling valves in GC)

Combination with autosamplers for high reproducible sample injection
# HPLC Columns

<table>
<thead>
<tr>
<th>Normal phase HPLC</th>
<th>Stationary phase</th>
<th>Mobile phase</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\text{Al}_2\text{O}_3$, $\text{SiO}_2$</td>
<td>hydrocarbons, iso-propanol</td>
<td>non-polar compounds (e.g. hydrocarbons, halohydrocarbons, ethers)</td>
</tr>
<tr>
<td>Reversed phase HPLC</td>
<td>$\text{SiO}_2-(\text{CH}_2)_n-\text{CH}_3$ ($n = 8$ – RP 8 or 18 – RP 18) “endcapped columns” = quantitative saturation of all OH groups by $-\text{CH}_3$</td>
<td>water, methanol, acetonitrile</td>
<td>polar compounds (e.g. alcohols, carbon acids)</td>
</tr>
</tbody>
</table>
# HPLC Detectors

<table>
<thead>
<tr>
<th>Name</th>
<th>Selectivity</th>
<th>Typical minimum detectable level [g/ml]</th>
<th>Linear dynamic range</th>
</tr>
</thead>
</table>
| UV-VIS detector/ Diode array detector<sup>1</sup> | - for larger organic molecules and transition metal compound which absorb UV-VIS light  
- time resolved recording of UV-VIS spectra, possibility of deconvolution of non-separated peaks | 5 \cdot 10^{-10}                      | 5 \cdot 10^4          |
| Fluorescence detector<sup>1</sup>        | - detects fluorescence radiation emitted by the sample compounds  
- specific for highly condensed organic molecules like PAH | 10^{-10} \ldots 10^{-9}                | \sim 10^3             |
| Refraction index detector                | - non-specific low-cost detector                                           | 5 \cdot 10^{-10}                      | 10^4                 |
| Electric conductivity detector           | - specific low-cost detector for compounds dissociated into ions (e.g. inorganic and organic salts, tensides, amino acids) | 10^{-8}                              | 10^3                 |
| Mass selective detector<sup>1</sup>      | - most selective detector for HPLC  
- strong requirements for the interface (transition from the high column pressure to vacuum inside the MSD)  
- high costs | no data available                                                           | 10^5                 |

<sup>1</sup> – suitable for gradient techniques
Special HPLC Techniques

Ion Chromatography/Ion Exchange Chromatography (IC/IEC):
- stationary phase: ion exchange resins
  (R-SO$_3^-$ or R-COO$^-$ for cation analysis, R-NH$_3^+$ or R-N(alkyl)$_3^+$ for anion analysis)
- mobile phase: aqueous solutions
  (diluted mineral acids for cation analysis,
  hydrogen carbonate buffer for anion analysis)
- detector: electric conductivity detector with pre-installed suppression column
- application: fast analysis of inorganic and organic salts in water
  (mainly alkali and alkaline earth metal salts)
- detection limit: 0.5 ppm

Capillary Electrophoresis (CE):
- operation of the column in an electrical field
- Movement of the ions is driven by electrical attraction.
- coupling with typical HPLC detectors (incl. MSD)
HPLC Application Examples

Biotechnological analysis: Amino acids

Pharmaceutical analysis: Basic drugs

Sample: Basic Drugs
Column: Aashipak ODP-50, 4 x 250 mm, 5 μm, (P/N: 799230P-584)
Mobile phase: A = Buffer pH 12, 1:19 diluted
B = Acetonitrile
Gradient: 10–70% B in 8 min
Flow rate: 1 ml/min
Temperature: 50°C
Detection: UV
Pub No.: 12-5

Sample: Amino acids (10 pmol each)
Column: Amino Acid (C18) column, 2.1 x 200 mm, 5 μm, (P/N: 79915AA-572)
Mobile phase: A = 20 mM sodium acetate, 0.018% TEA, pH 7.2, 0.3% THF
B = 100 mM sodium acetate, pH 7.2, acetonitrile, methanol (1/2/2)
Gradient: 0 to 60% B in 17 min
Flow rate: 0.45 ml/min
Temperature: 40°C
Detection: Fluorescence

Environmental analysis: Polycyclic aromatic hydrocarbons

Sample: PAH Standard
Column: LiChrospher PAH, 3.0 X 250 mm, 5 μm, (P/N: 79925FA-583)
Mobile phase: A = Water, B = Acetonitrile
Gradient: 0 min 50% B, 3 min 60% B, 15.4 min 100% B, 23.5 min 50% B
Flow rate: 0.6 ml/min
Temperature: 27°C
Detection: 254 nm
Quantitative Analysis

External standard
(1) Calibration for a known substance by injection of different known substance concentrations, calculation of a regression curve
(2) Injection of the sample with the unknown concentration, back calculation of the concentration by using the calibration function

Internal standard (elimination of sensitivity variations)
(1) Adding of a known equal amount of a substance which is not a part of the sample to each calibration sample and to the sample with the unknown concentration
(2) Normalization of the signal response (peak area or height) of all components on base of a constant signal of the external standard compound ($f = Y_{\text{norm}}/Y_{\text{real}}$)

Standard addition
(1) Injection of the sample with the unknown concentration
(2) Adding of known amounts of the substance which should be analyzed and performing a new analysis after each addition
(3) Calculation of the unknown concentration by setting $Y = 0$ for the concentration-response-function