Chromatographic separations

Chapter 26

The “stuff” you do before you analyze a “complex” sample
It is all about “Reducing Interferences”

<table>
<thead>
<tr>
<th>Method</th>
<th>Basis of Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Masking</td>
<td>Immobilization of interferent as a nonreactive complex</td>
</tr>
<tr>
<td>2. Mechanical phase separation</td>
<td></td>
</tr>
<tr>
<td>a. Precipitation and filtration</td>
<td>Difference in solubility of compounds formed</td>
</tr>
<tr>
<td>b. Distillation</td>
<td>Difference in volatility of compounds</td>
</tr>
<tr>
<td>c. Extraction</td>
<td>Difference in solubility in two immiscible liquids</td>
</tr>
<tr>
<td>d. Ion exchange</td>
<td>Difference in stability of reactants with an ion-exchange resin</td>
</tr>
<tr>
<td>3. Chromatography</td>
<td>Difference in rate of movement of a solute through a stationary phase</td>
</tr>
<tr>
<td>4. Electrophoresis</td>
<td>Difference in migration rate in an electrical field gradient</td>
</tr>
</tbody>
</table>
Chromatography basics

- Mobile and Stationary phase
- Retention - Migration
- Bands or zones
- Equilibrium!

- Column vs. planar
- Liquid vs. gas vs. SF
- High vs. low resolution
- Partition
- Adsorption
- Ion exchange
- Size exclusion
# Chromatography

A. Column Chromatography, B. Planar Chromatography

<table>
<thead>
<tr>
<th>General Classification</th>
<th>Specific Method</th>
<th>Stationary Phase</th>
<th>Type of Equilibrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid chromatography (LC)</td>
<td>Liquid-liquid, or partition</td>
<td>Liquid adsorbed on a solid</td>
<td>Partition between immiscible liquids</td>
</tr>
<tr>
<td>(mobile phase: liquid)</td>
<td>Liquid-bonded phase</td>
<td>Organic species bonded to a solid surface</td>
<td>Partition between liquid and bonded surface</td>
</tr>
<tr>
<td></td>
<td>Liquid-solid, or adsorption</td>
<td>Solid</td>
<td>Adsorption</td>
</tr>
<tr>
<td></td>
<td>Ion exchange</td>
<td>Ion-exchange resin</td>
<td>Ion exchange</td>
</tr>
<tr>
<td></td>
<td>Size exclusion</td>
<td>Liquid in interstices of a polymeric solid</td>
<td>Partition/sieving</td>
</tr>
<tr>
<td>Gas chromatography (GC)</td>
<td>Gas-liquid</td>
<td>Liquid adsorbed on a solid</td>
<td>Partition between gas and liquid</td>
</tr>
<tr>
<td>(mobile phase: gas)</td>
<td>Gas-bonded phase</td>
<td>Organic species bonded to a solid surface</td>
<td>Partition between liquid and bonded surface</td>
</tr>
<tr>
<td></td>
<td>Gas-solid</td>
<td>Solid</td>
<td>Adsorption</td>
</tr>
<tr>
<td>Supercritical-fluid chromatography (SFC)</td>
<td></td>
<td>Organic species bonded to a solid surface</td>
<td>Partition between supercritical fluid and bonded surface</td>
</tr>
</tbody>
</table>
Column Chromatography

Chromatogram

Dilution & Peak broadening
Chromatography: Peak separations
Chromatography: Peak Resolution

- Poor resolution
- More separation
- Less band spread
Chromatography:
Distribution Constant (recommended by IUPAC)
(old term: partition coefficient)

A mobile ↔ A stationary

\[ K_c = \frac{C_S}{C_M} \]

\[ C_S = \frac{n_s}{V_s}, \quad C_M = \frac{n_M}{V_M} \]

\( K \sim \text{constant} \implies \text{linear chromatography} \)

\( >>>>K >>>> \) Retention in the stationary phase → Retention times

How to manipulate \( K \)?
Chromatography  Retention Times

\[ t_M = \text{retention time of mobile phase (dead time)} \]
\[ t_R = \text{retention time of analyte (solute)} \]
\[ t_S = \text{time spent in stationary phase (adjusted retention time)} \]
\[ L = \text{length of the column} \]
Chromatography: Velocities
Linear rate of solute migration!

Velocity = distance/time \rightarrow length of column/retention times

Velocity of solute: \[ \bar{v} = \frac{L}{t_R} \]
Velocity of mobile phase: \[ \mu = \frac{L}{t_M} \]
Chromatography
Velocity/Retention time and Kc

\[
\bar{v} = \mu \times \text{fraction of time in mobile phase}
\]

\[
\bar{v} = \mu \times \frac{\text{moles of solute in mobile phase}}{\text{total moles of solute}}
\]

\[
\bar{v} = \mu \times \frac{c_M V_M}{c_M V_M + c_S V_S}
\]
Chromatography
Velocity Relationships

\[ \bar{v} = \mu \times \frac{c_M V_M}{c_M V_M + c_S V_S} \]

\[ \bar{v} = \mu \times \frac{1}{1 + c_S V_S / c_M V_M} \]

\[ K = \frac{c_S}{c_M} \quad \text{Distribution Constant} \]

\[ \bar{v} = \mu \times \frac{1}{1 + K V_S / V_M} \]
Chromatography

Retention Factor: are we there yet?

\[
\bar{v} = \mu \times \frac{1}{1 + K \frac{V_s}{V_M}}
\]

\[
k_A = K_A \frac{V_s}{V_M} \quad \text{(Retention Factor)}
\]

\[
\bar{v} = \mu \times \frac{1}{1 + k_A}
\]

\[
\frac{L}{t_R} = \frac{L}{t_M} \times \frac{1}{1 + k_A}
\]

\[
k_A = \frac{t_R - t_M}{t_M} \quad \text{Adjusted retention time}
\]
Relative retention time:

\[ \text{RRT} = \frac{t_R}{t_{Rs}} \]

\[ t_{Rs} = \text{retention time of internal standard} \]
Chromatography
Selectivity Factor: can you separate from your neighbor

B retained more than A $\rightarrow \alpha > 1$

$$\alpha = \frac{K_B}{K_A}$$ Distribution Constant

$$\alpha = \frac{k_B}{k_A}$$ Retention factor

$$k_A = \frac{(t_R)_A - t_M}{t_M} \text{ and } k_B = \frac{(t_R)_B - t_M}{t_M}$$

$$\alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M}$$ Retention time
Chromatography
Column Efficiency - Theoretical Plates
Plate and Rate Theories

\[ H = \text{plateheight} \]
\[ N = \text{number of plates} \]
\[ N = \frac{L}{H} \]
\[ H = \frac{\sigma^2}{L} \]

\( L \) = length of column packing
\( \sigma \) \( \rightarrow \) standard deviation \( \sigma^2/L \) \( \rightarrow \) variance per unit length.
Chromatography
Relation between column distance and retention times

$L = \text{column length (distance)}$

$\sigma = \text{standard deviation in distance}$

$t_R = \text{retention time}$

$\tau = \text{standard deviation in time}$

$$\frac{\sigma}{L} = \frac{\tau}{t_R}$$

$$\tau = \frac{\sigma}{L/t_R}$$
Chromatography
Relation between column distance and retention times

\[
\frac{\sigma}{L} = \frac{\tau}{t_R}
\]

\[
\sigma = \frac{\tau L}{t_R}
\]

\[
W = 4\tau
\]

\[
\sigma = \frac{WL}{4t_R}
\]

\[
H = \frac{\sigma^2}{L} = \frac{W^2 L}{16t_R^2}
\]

Tangent at Inflection point

\(~96\% ~\pm ~2\tau\)

Analyte profile at end of packing

Number of molecules

Detector signal

Time
Chromatography
Determining the Number of Theoretical Plates

\[ N = 16 \left( \frac{t_R}{W} \right)^2 \]

\[ N = 5.54 \left( \frac{t_R}{W_{1/2}} \right)^2 \]
Summary of Plate Theory

- Successfully accounts for the peak shapes and rate of movement
- Does not account for the “mechanism” causing peak broadening
- No indication of other parameters’ effects
- No indication for adjusting experimental parameters
Rate Theory

- Zone broadening is related to Mass Transfer processes
### Column Efficiency

**Kinetic variables**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Symbol</th>
<th>Usual Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear velocity of mobile phase</td>
<td>$u$</td>
<td>cm s$^{-1}$</td>
</tr>
<tr>
<td>Diffusion coefficient in mobile phase*</td>
<td>$D_M^{**}$</td>
<td>cm$^2$ s$^{-1}$</td>
</tr>
<tr>
<td>Diffusion coefficient in stationary phase*</td>
<td>$D_S$</td>
<td>cm$^2$ s$^{-1}$</td>
</tr>
<tr>
<td>Retention factor (Equation 26-12)</td>
<td>$k$</td>
<td>unitless</td>
</tr>
<tr>
<td>Diameter of packing particles</td>
<td>$d_p$</td>
<td>cm</td>
</tr>
<tr>
<td>Thickness of liquid coating on stationary phase</td>
<td>$d_f$</td>
<td>cm</td>
</tr>
</tbody>
</table>

TABLE 26-2 Variables That Influence Column Efficiency
Zone Broadening

Flow Rate of Mobile Phase

Liquid chromatography

Gas chromatography

Note the differences in flowrate and plates height scales

Why GC normally has high H, but also high overall efficiency?
Zone Broadening

Kinetic Processes

TABLE 26-3 Processes That Contribute to Band Broadening

<table>
<thead>
<tr>
<th>Process</th>
<th>Term in Equation 26-23</th>
<th>Relationship to Column* and Analyte Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple flow paths</td>
<td>$A$</td>
<td>$A = 2\lambda d_p$</td>
</tr>
<tr>
<td>Longitudinal diffusion</td>
<td>$B/u$</td>
<td>$B/u = 2\gamma D_M$</td>
</tr>
<tr>
<td>Mass transfer to and from stationary phase</td>
<td>$C_S u$</td>
<td>$C_S u = \frac{f(k)d_i^2}{D_S} u$</td>
</tr>
<tr>
<td>Mass transfer in mobile phase</td>
<td>$C_M u$</td>
<td>$C_M u = \frac{f'(k)d_p^2}{D_M} u$</td>
</tr>
</tbody>
</table>

Van - Deemter Equation

$\lambda$ and $\gamma$ are constants that depend on quality of the packing.

$B$ is coefficient of longitudinal diffusion.

$C_S$ and $C_M$ are coefficients of mass transfer in stationary and mobile phase, respectively.

$H = A + B/\mu + (C_S + C_M)\mu$
Zone Broadening

Kinetic Processes

<table>
<thead>
<tr>
<th>Process</th>
<th>Term in Equation 26-19</th>
<th>Relationship to Column* and Analyte Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple flow paths</td>
<td>$A$</td>
<td></td>
</tr>
<tr>
<td>Longitudinal diffusion</td>
<td>$B/\mu$</td>
<td></td>
</tr>
<tr>
<td>Mass transfer to and from liquid stationary phase</td>
<td>$C_{Su}$</td>
<td></td>
</tr>
<tr>
<td>Mass transfer in mobile phase</td>
<td>$C_{Mu}$</td>
<td></td>
</tr>
</tbody>
</table>

Van - Deemter Equation

$$H = A + \frac{B}{\mu} + (C_S + C_M)\mu$$
Eddy Diffusion: band broadening process results from different path lengths passed by solutes.

1. Directly proportional to the diameters of packing
2. Offset by ordinary diffusion
3. Lower mobile-phase velocity, smaller eddy diffusion

Stagnant pools of mobile phase retained in stationary phase.
Zone Broadening
Longitudinal Diffusion

- The higher the $\mu$, the smaller the $H$
- Much smaller in LC than in GC
Zone Broadening

Mass Transfer between Phases

- Slow equilibrium of solute between mobile and stationary phases
- Time is required for solute molecules to diffuse from the interior of these phases to their interface where transfer occurs
Zone Broadening

Longitude vs. Mass Transfer

- **Longitude**
  - Parallel to the flow
  - Inversely proportional to the flow of the mobile phase

- **Mass Transfer**
  - Diffusion tends to be right angles to the flow
  - The faster the mobile phase moves, the larger the band broadening
Chromatography

Resolution

$$R_s = \frac{\Delta Z}{W_A/2 + W_B/2}$$

$$R_s = \frac{2\Delta Z}{W_A + W_B}$$

$$R_s = \frac{2[(t_{R_B}) - (t_{R_A})]}{W_A + W_B}$$
Chromatographic Separations with a twist
# Chromatographic Definitions

<table>
<thead>
<tr>
<th>Name</th>
<th>Symbol of Experimental Quantity</th>
<th>Determined From</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migration time, nonretained species</td>
<td>$t_M$</td>
<td>Chromatogram (Figure 26-6)</td>
</tr>
<tr>
<td>Retention times, species A and B</td>
<td>$(t_R)_A, (t_R)_B$</td>
<td>Chromatogram (Figure 26-6)</td>
</tr>
<tr>
<td>Adjusted retention time, species A</td>
<td>$(t'_R)_A$</td>
<td>$(t'_R)_A = (t_R)_A - t_M$</td>
</tr>
<tr>
<td>Peak widths, species A and B</td>
<td>$W_A, W_B$</td>
<td>Chromatogram (Figure 26-6)</td>
</tr>
<tr>
<td>Length of column packing</td>
<td>$L$</td>
<td>Direct measurement</td>
</tr>
<tr>
<td>Flow rate</td>
<td>$F$</td>
<td>Direct measurement</td>
</tr>
<tr>
<td>Volume of stationary phase</td>
<td>$V_S$</td>
<td>Packing preparation data</td>
</tr>
<tr>
<td>Concentration of analyte in mobile and stationary phases</td>
<td>$c_M, c_S$</td>
<td>Analysis and preparation data</td>
</tr>
</tbody>
</table>
# Chromatographic Relationships

<table>
<thead>
<tr>
<th>Name</th>
<th>Calculation of Derived Quantities</th>
<th>Relationship to Other Quantities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear mobile-phase velocity</td>
<td>$u = L/t_M$</td>
<td></td>
</tr>
<tr>
<td>Volume of mobile phase</td>
<td>$V_M = t_M F$</td>
<td></td>
</tr>
<tr>
<td>Retention factor</td>
<td>$k' = (t_R - t_M)/t_M$</td>
<td>$k' = \frac{K V_S}{V_M}$</td>
</tr>
<tr>
<td>Distribution constant</td>
<td>$K = \frac{k' V_M}{V_S}$</td>
<td>$K = \frac{c_S}{c_M}$</td>
</tr>
<tr>
<td>Selectivity factor</td>
<td>$\alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M}$</td>
<td>$\alpha = \frac{k'_B}{k'_A} = \frac{K_B}{K_A}$</td>
</tr>
<tr>
<td>Resolution</td>
<td>$R_s = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$</td>
<td>$R_s = \frac{\sqrt{N}}{4} \left( \frac{\alpha - 1}{\alpha} \right) \left( \frac{k'_B}{1 + k'_B} \right)$</td>
</tr>
<tr>
<td>Number of plates</td>
<td>$N = 16 \left( \frac{t_R}{W} \right)^2$</td>
<td>$N = 16 R_s^2 \left( \frac{\alpha}{\alpha - 1} \right)^2 \left( \frac{1 + k'_B}{k'_B} \right)^2$</td>
</tr>
<tr>
<td>Plate height</td>
<td>$H = L/N$</td>
<td></td>
</tr>
<tr>
<td>Retention time</td>
<td>$(t_R)_B = \frac{16 R_s^2 H}{u} \left( \frac{\alpha}{\alpha - 1} \right)^2 \left( 1 + k'_B \right)^3}{(k'_B)^2}$</td>
<td></td>
</tr>
</tbody>
</table>
Quantitative Analysis

- Peak areas
- Peak height
- Calibration and standards
- Internal Standard method
Summary

- Relate to column chromatography
- Retention times
- Velocities of mobile and component
- Height equivalent of theoretical plates
- Peak or zone broadening
- Resolution