Chapter 26 – Introduction to Separation Science (Chromatography)

Read: pp. 762-783
Problems: 26-2, 5, 6, 7, 10

Separation science is a key aspect of most every analytical methodology! Most often, a real world sample contains many analytes that are responsive to the particular perturbation (e.g., light), and most analytical methods do not offer sufficient selectivity for all the analytes that might be present.

Therefore, most methods involve analyte separation first, followed by detection.
Separation Principle and The Chromatograph

**FIGURE 28-1** (a) Diagram showing the separation of a mixture of components A and B by column elution chromatography. (b) The output of the signal detector at the various stages of elution shown in (a).

(a)

(b)

Detector signal

Time
Imagine that you are tasked with investigating how electrical impulses are transferred from nerve cell to cell in the brain of Parkinson’s patients and how these signals are affected by changing concentrations of one primary neurotransmitter, dopamine.

Need to separate the aromatic amines so that each can be individually detected.

Polar, water soluble, aromatic molecules

Separation and Detection method must be selected!
# Types of Separation Methods

Mobile Phase + Stationary Phase

## TABLE 26-1 Classification of Column Chromatographic Methods

<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>
Basics of the Separation

Mobile phase and Stationary phase

The longer the analytes (solutes) spend interacting with the stationary phase, the better the separation efficiency is but the broader (in time) the peaks get.
Differential Interaction of Solute with Stationary and Mobile Phases

**FIGURE 26-2** Concentration profiles of solute bands A and B at two different times in their migration down the column in Figure 26-1. The times $t_1$ and $t_2$ are indicated in Figure 26-1.
Asymmetric Peak Shapes

FIGURE 26-5 Illustration of fronting and tailing in chromatographic peaks.
Basics of the Separation

\[ N = \frac{L}{H} \] (efficiency)

\[ N = \text{number of plates} \]
\[ L = \text{column length (cm)} \]
\[ H = \text{plate height (cm/plate)} \]

A pseudo-equilibrium is established on the column as the analyte (solute) develops a distribution within the mobile phase and the stationary phase.

\[ K_{\text{eq}} \text{ (partition coefficient)} = \frac{C_{\text{stat}}}{C_{\text{mobile}}} \]

**Figure 26-5** Definition of plate height \( H = \frac{\sigma^2}{L} \).
Basics of the Separation Method

\[ k' \text{ (capacity factor)} = \frac{(t_r - t_m)}{t_m} \]

\[ \alpha \text{ (selectivity factor)} = \frac{k'_b}{k'_a} \]

\[ u \text{ (mobile phase flow vel., cm/s)} = \frac{L}{t_m} \]

\[ K_{eq} \text{ (distribution coeff.)} = \frac{(k'V_m)}{V_s} \]

\[ N \text{ (plate number)} = \frac{L}{H} = 16 \left( \frac{t_r}{W} \right)^2 \]

\[ R_s \text{ (resolution)} = 2\left[ (t_{r_b} - t_{r_a}) / (W_b + W_a) \right] \]

**Figure 26-6** Determination of the standard deviation \( \tau \) from a chromatographic peak: \( W = 4\tau \).

**TABLE 26-2** Variables That Affect Column Efficiency

<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\cdot s^{-1}</td>
</tr>
<tr>
<td>Diffusion coefficient in mobile phase*</td>
<td>( D_M )</td>
<td>cm^{2}\cdot s^{-1}</td>
</tr>
<tr>
<td>Diffusion coefficient in stationary phase*</td>
<td>( D_S )</td>
<td>cm^{2}\cdot s^{-1}</td>
</tr>
<tr>
<td>Retention factor (Equation 26-8)</td>
<td>( k' )</td>
<td>unitless</td>
</tr>
<tr>
<td>Diameter of packing particle</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>

*Increases as temperature increases and viscosity decreases.
## Chromatographic Experimental Quantities

**TABLE 26-4** Important Chromatographic Experimental Quantities and Relationships

<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>
# Separation Figures of Merit

## TABLE 26-5 Important Derived Quantities and 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>$k' = \frac{KV_S}{V_M}$</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>$N = 16R_s^2 \left( \frac{\alpha}{\alpha - 1} \right)^2 \left( \frac{1 + k'_B}{k'_B} \right)^2$</td>
</tr>
<tr>
<td>Plate height</td>
<td>$H = L/N$</td>
<td></td>
</tr>
<tr>
<td>Retention time</td>
<td>$(t_R)_B = \frac{16R_s^2H}{u} \left( \frac{\alpha}{\alpha - 1} \right)^2 \left( 1 + k'_B \right)^3 \left( \frac{1}{(k'_B)^2} \right)$</td>
<td></td>
</tr>
</tbody>
</table>
Factors Influencing Peak Widths

Retention time → Peak width → Resolution → Efficiency

What one wants is the shortest retention time that gives adequate resolution of all closely eluting components!

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

One wants a small value for H!

*Figure 26-7* Effect of mobile-phase flow rate on plate height for (a) liquid chromatography and (b) gas chromatography.
Factors Influencing Peak Widths

What factors make $H$ large?

$$H = A + \frac{B}{u} + Cu$$

*Figure 26-8* Typical pathways of two molecules during elution. Note that distance traveled by molecule 2 is greater than that traveled by molecule 1. Thus, molecule 2 would arrive at $B$ later than molecule 1.

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**TABLE 26-3** Kinetic Processes That Contribute to Peak Broadening

<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>$A = 2\lambda d_P$</td>
</tr>
<tr>
<td>Longitudinal diffusion</td>
<td>$B/u$</td>
<td>$B/u = \frac{2\gamma D_M}{u}$</td>
</tr>
<tr>
<td>Mass transfer to and from liquid stationary phase</td>
<td>$C_{SU}$</td>
<td>$C_{SU} = \frac{f_S(k')d_L^2}{D_S}u$</td>
</tr>
<tr>
<td>Mass transfer in mobile phase</td>
<td>$C_{MU}$</td>
<td>$C_{MU} = \frac{f_M(k')d_P^2}{D_M}u$</td>
</tr>
</tbody>
</table>

* $u$, $D_L$, $D_M$, $d_L$, $d_P$, $k'$ are as defined in Table 26-2.
  $f(x)$ = function of $x$.
  $\lambda$, $\gamma$: constants that depend on the quality of the packing.
  $B$: coefficient of longitudinal diffusion.
  $C_S$, $C_M$: coefficients of mass transfer in stationary and mobile phases, respectively.
Factors Influencing Peak Widths

$C_s$ mass transport to and from stationary phase

$C_m$ mass transport in mobile phase

\[
C_s \propto \frac{d_f}{D_s} \quad \quad \quad C_m \propto \frac{d_p^2}{D_m}
\]

Migration time

Remember: the stationary phase is pretty uniform in terms of its structure and chemistry. Therefore, differential rates of migration can be established
Factors Influencing Peak Widths

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

**Figure 26-9** A van Deemter plot for a packed liquid chromatographic column. The points on the upper curve are experimental. The contributions of the various rate terms are shown by the lower curves: \( A \), multipath effect; \( B/u \), longitudinal diffusion; \( Cu \), mass transfer for both phases. (From E. Katz, K. L. Ogan, and R. P. W. Scott, J. Chromatogr., 1983, 270, 51. With permission.)

- **Mass transport factor**
- **Tortuous pathways**
- **Longitudinal diffusion**

Remember \( N = L/H \)!
Factors Influencing Peak Widths

The plate height, $H$, depends on the particle size!! A and C terms of Van deemter equation increase with particle size.

Figure 26-10  Effect of particle size on plate height. The numbers to the right are particle diameters.  (From J. Boheman and J. H. Purnell, in Gas Chromatography, 1958, D. H. Desty, Ed. New York: Academic Press, 1958. With permission of Butterworths, Stoneham, MA.)
Resolution (Time) in Chromatograms

Adjustments of the stationary phase material and properties, the linear flow velocity of the mobile phase, and the mobile phase composition are all varied to optimize the separation.

Desired large \( N \), high resolution and short analysis time!

*Figure 26-11*  Separations at three resolutions. Here, \( R_T = 2\Delta Z/(W_A + W_B) \).
The General Elution Problem

To solve this problem, the separation conditions are often not kept constant during the entire run, but rather are altered at some point during the run. Typical separations often begin with one set of conditions and end with a another set.