Chromatographic resolution:

The resolution of any technique that disperses analyte species in time, distance, mass, or any other parameter is the relationship between the distance between adjacent peaks and their widths.

In the case of column chromatography, the distance between peaks is measured in time, in terms of retention times.

\[
R_s = \frac{2[t_{R,B} - t_{R,A}]}{W_A + W_B}
\]
Chromatographic resolution is defined as the ratio of the difference of the retention times of two components to the sum of the peaks widths at the base:

\[
R_s = \frac{2|t_{R,B} - t_{R,A}|}{W_A + W_B}
\]

W at the base, as determined by lines drawn from the tangents of the inflection points, equals ±2σ (4σ)

Sometimes it is easier to use the width of the peak at half height. Then \(W_{h/2} = 2.35\sigma\) rather than \(W = 4\sigma\).
Relationship to retention factors, $k$, and selectivity factors, $\alpha$:

$$R_S = \frac{\sqrt{N}}{4} \left( \frac{\alpha - 1}{\alpha} \right) \left( \frac{k_B}{1 + k_B} \right)$$

where $k_B$ is the retention factor of the later eluting species.

$$\frac{V_S K_B}{V_M} = \frac{t_R - t_M}{t_M} = \frac{t_s}{t_M} = k_B = \text{retention factor}$$

$$\alpha = \frac{k_B}{k_A} = \frac{K_B}{K_A} = \text{selectivity factor}$$

The efficiency needed to realize a given resolution:

$$N = 16 R_S^2 \left( \frac{\alpha}{\alpha - 1} \right)^2 \left( \frac{1 + k_B}{k_B} \right)^2$$
Elution time required for a given resolution, H, u, k_B, and α:

\[ t_{R,B} = \frac{16R_S^2H}{u} \left( \frac{\alpha}{\alpha - 1} \right)^2 \frac{1 + k_B}{k_B^2} \]

Example

Liquid chromatographic data for a) caffeine, b) aspartate, c) benzoate (2 mL/100 mL)

What linear flow rate was used to collect this spectrum? \( L/t_M = 0.24 \text{ cm/s} \)
what is H for this column?

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

\[ \alpha = \frac{462 - 41.5}{188 - 41.5} = 2.87 \]

\[ k_{benz.} = \frac{t_{Rbenz.} - t_M}{t_M} = \frac{462 - 41.5}{41.5} = 10.1 \]

\[ R_S = \frac{2[t_{R,B} - t_{R,A}]}{W_A + W_B} = \frac{2(462 - 188)}{(11 + 20)} = 17.6 \]

\[ N = 16R_S^2 \left( \frac{\alpha}{\alpha - 1} \right)^2 \left( \frac{1 + k_B}{k_B} \right)^2 \]

plugging in the values for \( R_S, \alpha, k_B \) and solving for \( N \) gives \( N = 14,100 \)

\[ H = 10 \text{ cm}/14100 = 7.1 \times 10^{-4} \text{ cm} \]

or

\[ t_{R,B} = \frac{16R_S^2 H}{u} \left( \frac{\alpha}{\alpha - 1} \right)^2 \left( \frac{1 + k_B}{k_B} \right)^3 \]

plug in values for \( t_{R,B}, R_S, u, k_B, \) and \( \alpha \) and solving for \( H \) gives \( H = 7.1 \times 10^{-4} \text{ cm} \)
At what retention time will caffeine and benzoate be separated with a resolution of 1.5?

\[ t_{R,B} = \frac{16R_S^2 H}{u} \left( \frac{\alpha}{\alpha - 1} \right)^2 \frac{(1 + k_B)^3}{k_B^2} \]

\[ t_{R,B} = \frac{16(1.5)^2(7.1 \times 10^{-4})}{0.24} \left( \frac{2.87}{2.87 - 1} \right)^2 \frac{(1 + 10.1)^3}{(10.1)^2} = 3.36s \]

so, these two components are nearly baseline separated in the column as early as 3.36 s after injection…
Caffeine

\[ K_a = 10^{-7}, \quad pK_a = 7 \]

Aspartame

\[ pK_a^{NH_3^+} = 9.6 \]
\[ pK_a^{COOH^-} = 3.0 \]

Benzolic Acid

\[ pK_a = 4.2 \]

In Exp. 10, the mobile phase was a mixture of acetonitrile (CH\(_3\)C\(=\)N) 400 mL \(\setminus\) 7 diluted with glacial acetic acid 2.3 mL \(\setminus\) H\(_2\)O to 2 L with NaOH added to give pH 4.2.

At this pH caffeine is +1

aspartame is a zwitterion (±)

benzoic acid = benzoate 0 and -1 charges
Liquid chromatographic data for a) caffeine, b) aspartate, c) benzoate (2 mL/100 mL)
Caffeine likes the aqueous phase more than the C-18 stationary phase and is eluted first.

Aspartame has one end which is in the zwitter ion form, and is attracted to the mobile phase. The other end of the molecule is attracted to the C-18 phase.

Benzonic acid = benzoate ion (species in rapid equilibria always elute as a single peak)

The aromatic group is attracted to the C-18 phase and hence is the last to elute.

But why does the aspartame peak show such a non-Gaussian behavior?
Hydrolysis of aspartame on the column is likely to occur. Degradation on column can also give rise to peak asymmetry.
Goal: maximum separation in minimum time

mutually exclusive requirements – requires a compromise

realistic objective: satisfactory separation in acceptable time

\[ R_s = \frac{\sqrt{N}}{4} \left( \frac{\alpha - 1}{\alpha} \right) \left( \frac{k_B}{1 + k_B} \right) \]

\[ t_{R,B} = \frac{16R_s^2H}{u} \left( \frac{\alpha}{\alpha - 1} \right) \left( \frac{1 + k_B}{k_B^2} \right) \]

factors that affect efficiency: N,H

factors that affect selectivity: \( \alpha \)
(solute properties)

factors that affect retention: \( k_B \)
(solute and column properties)
Variables that affect $N,H$: via effects on $A$, $B/u$, $Cu$, and the relationship between these factors (e.g., the van Deemter equation)

<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 (see Equation 30-18)</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>$d_l$</td>
<td>cm</td>
</tr>
</tbody>
</table>

*Increases as temperature increases and viscosity decreases.

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Variables that affect retention factor:
temperature in gas chromatography
solvent composition in liquid chromatography
stationary phase and its thickness

$k$ affects both $R_S$ and $t_R$, for this reason, $k_B$ values of 1-5 are generally preferred – larger $k_B$ values increase retention time with relatively little gain in resolution
Factors that affect $\alpha$:
$$\alpha = \frac{k_B}{k_A} = \frac{K_B}{K_A} = \text{selectivity factor}$$

note that factors affecting $k_B$ can also affect $\alpha$, so it is not always straightforward to change $\alpha$ without changing $k_B$ to an unacceptable value.

tactics:
- mobile phase composition (static (isocratic), dynamic (gradient))
- column temperature (GC) (isothermal, programmed)
- stationary phase
- chemical additives
We added $I^-$ to aqueous phase in solvent extraction to change the partitioning between organic/aqueous phases for $I_2$. (we made $I_2$ more polar)

Analogous strategies are used in LC:

Ion pairing strategies executed with reverse-phase LC

organic salt added to mobile phase to interact with ionized groups on analyte to alter partitioning

1. niacinamide, 2. pyridoxine, 3. riboflavin, 4. thiamine