

Separation Theory

Analyze Complex mixtures	If analyte produce overlapping signals
Process of unmixing a sample	Input energy Analyte are diluted

Requirements

Stationary Phase (SP)	Fixed in column Interacts with analyte
Mobile Phase (MP)	Moves through/over SP Carries analyte
Interactions	No interaction with SP ▶ Travel same speed as MP ▶ No retention = No separation Interaction with SP ▶ Analyte are retained → Dispersion ▶ Part time in SP ($v=0$) and MP (same speed) All analyte spends same amount of time in MP but diff. time in SP

Fundamental Processes

Retention	Peaks located in chromatogram
	Analyte interaction with column ▶ stationary phase: strongint. = slow rate Control by thermodynamic property ▶ alter property = alter retention Example: ▶ Temperature (GC) ▶ MP (LC) ▶ SP ▶ Analyte

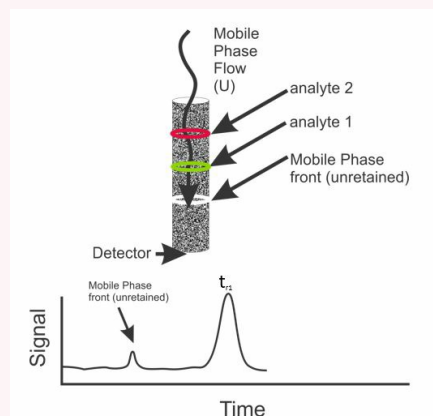
Fundamental Processes (cont)

Dispersion	Band Broadening ▶ peak width ▶ how dilute Ex: ↑ Dispersion = ↑ Intensity = ↑ [Analyte] Depends on structure of column ▶ ↑ Analyte mix = ↑ Dispersion Depends on diffusion of analyte ▶ ↑ Diffusion Coefficient = ↑ Dispersion Depends on total time in column ▶ ↑ Time ↑ Diffusion = ↑ Dispersion
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Separation Process

Occurs in tube/plate (TLC)
Drive MP through column ▶ consistent velocity ▶ Use a pump (LC) → HPLC ▶ Use capillary action (TLC) → Dip plate in MP ▶ Use gas pressure (GC) → Store MP in HP-cylinder + attach to gas regulator
Introduce sample at top of column
Allow MP to drive sample through/over SP
Detector at end (emerges vs. time)

Process



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Retention

Measure Retention (K')

Variables

- ▶ L=Length of column
- ▶ U= MP velocity
- ▶ V = Analyte velocity
- ▶ t_m = retention time of MP
- ▶ t_r =retention time of retained species
- ▶ K=distribution constant
- ▶ C_s = [Analyte] in SP
- ▶ C_m = [Analyte] in MP

t_r → use to identify analyte

Simple matrix → $1 < K' < 10$

Complex matrix → $0.5 < K' < 20$

K'

- ▶ Determined by chromatogram
- ▶ Controlled by equilibrium
- ▶ Judge separation by the last peak retention value

Control Retention

Connect to K

Control by thermodynamic property

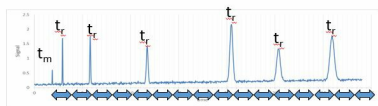
- ▶ Adjust temperature
- ▶ Adjust type of MP
- ▶ Adjust "strength" of MP/SP
- ▶ Add additives to MP → interact with analyte, SP, MP
- ▶ Velocity of MP does not alter retention

Retention Equations

$$\bar{U} = \frac{L}{t_m} \quad \bar{v} = \bar{U} \frac{1}{(1 + k')}$$

$$\bar{v} = \frac{L}{t_r} \quad \bar{v} = \frac{L}{t_r} = \frac{L}{t_m} \frac{1}{(1 + k')}$$

$$K = \frac{C_s}{C_m} \quad k' = \frac{t_r - t_m}{t_m} = K \frac{V_s}{V_m}$$



Efficiency

Quantify Efficiency

Treat chromatographic peaks like "Gaussian" peaks

Mean = Retention time

Quantify width peak

- ▶ standard deviation
- ▶ peak width

Smaller width = better efficiency

Narrow peaks = Good efficiency

- ▶ Clear separation

Broad peaks = Poor efficiency

- ▶ Overlapping

Peak Shapes

Sample volume ~ 1% column volume

Various processes in column spread into larger volume

- ▶ Often significant > starting volume
- ▶ Ex: Inj. volume = 25uL and detection volume = 200uL

Desirable

- ▶ Narrow peaks and small volume

"Gaussian Peaks"

- ▶ Peak could emerge with neighbor peak
- ▶ dilution can form broadening

Measure Efficiency

Variables

- ▶ N = # of theoretical plate
- ▶ H = Height of theoretical plate (HETP)
- ▶ L= Length of column
- ▶ W = peak width at baseline
- ▶ σ = Standard deviation (unit of length)

Desirable

- ▶ ↑ N = ↓ H = ↓ σ



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Efficiency (cont)

W range = -2τ to $+2\tau$

N should be consistent

- ▶ t_r and σ scale with each other

If Baseline not Accessible

Baseline peak width cannot be measured

- ▶ nearby overlapping peaks

Use upper portion of peak that is undistorted

- ▶ Use full-width at half maximum (FWHM)
- ▶ establish SD

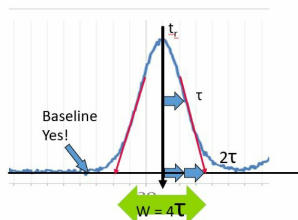
$W_{1/2} \neq 1/2 W$

"Gaussian Peak" At W

$$\tau = \frac{\sigma}{v} = \frac{\sigma}{L/t_r} = \frac{W}{4}$$

$$\sigma = \frac{LW}{4t_r} = \frac{W}{4}$$

$$N = \frac{L}{H} = 16 \left(\frac{t_r}{W} \right)^2 = \left(\frac{t_r}{\sigma} \right)^2 \quad H = \frac{\sigma^2}{L}$$



Band Broadening

Occurrences

Low efficiency

- ▶ Not fully separated peaks
- ▶ Interferences

Dispersion is independent of retention

Van

Deemeter

Overview

A-Term

- ▶ Associate with multiple flow paths through column
- ▶ Each unique distance
- ▶ Result in variety of times to transit column

B-Term

- ▶ Associate with longitudinal diffusion of analyte
- ▶ Some analyte will arrive sooner/later
- ▶ Depends on magnitude + direction of net diffusion during t_r

C-Term

- ▶ Split into 2 sub-terms
- ▶ Relate to reality that chromatogram is carried out in non-equilibrium state
- ▶ Analyte in Mp will be out of equilibrium with those in SP (vice versa)
- ▶ Some analyte will arrive at detector earlier or later than true equilibrium would predicted

Van

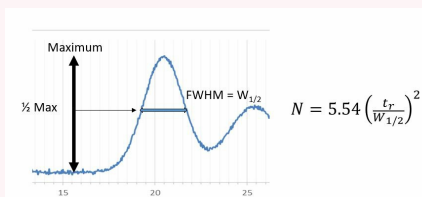
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Graph

Produce the overall curve with distinct minimum

- ▶ Corresponding to N_{max} and fixed L

Full-Width at Half Maximum



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Band Broadening (cont)

Overall Plate Height:

- ▶ Equation: $H = A + B/U + (C_s + C_m)U$
- ▶ Sum of 4 components (red line)

A-Term:

- ▶ Constant (purple line)

B/U-Term:

- ▶ Varies as $1/U$ (pink line)

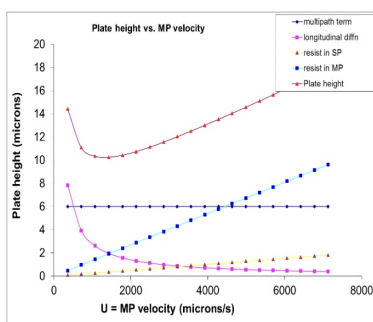
$C_s U$ -Term:

- ▶ Linear increasing (blue line)

$C_m U$ -Term:

- ▶ Linear increasing (yellow line)

Van Deemter Graph (copy)



$$H = A + \frac{B}{U} + (C_s + C_m)U$$

$$A = 2\lambda d_p \quad \frac{B}{U} = \frac{2\gamma D_m}{U}$$

$$C_m U = \frac{f_m(k')d_p^2}{D_m} U \quad C_s U = \frac{f_s(k')d_f^2}{D_s} U$$

A-Term: Multipath Band Broadening

All molecules start at top of column

As they move down

- ▶ Follow different paths through particles
- ▶ Irrespective of interaction with SP

Range of paths depends on size of particles

- ▶ ↑ Size = ↓ # of paths = ↑ Path length

Depends on how "packed: the bed is

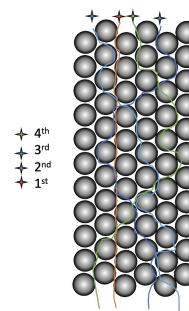
- ▶ Crack, voids, etc

A-Term: Multipath Band Broadening (cont)

Equation:

- ▶ $H_{A-term} = 2\lambda d_p$
- ▶ λ = quality/tortuosity factor
- ▶ ~0.5-0.6 (packed column)
- ▶ FSOT less

A-Term Diagram



B/U-Term: Longitudinal Diffusion

All molecules start at top of column

As they move down

- ▶ Molecules moves away from each other
- ▶ Process continues as long as they remain in column

Dispersion in all 3 directions

- ▶ Only longitudinal dispersion impacts peak width (↑ and ↓)

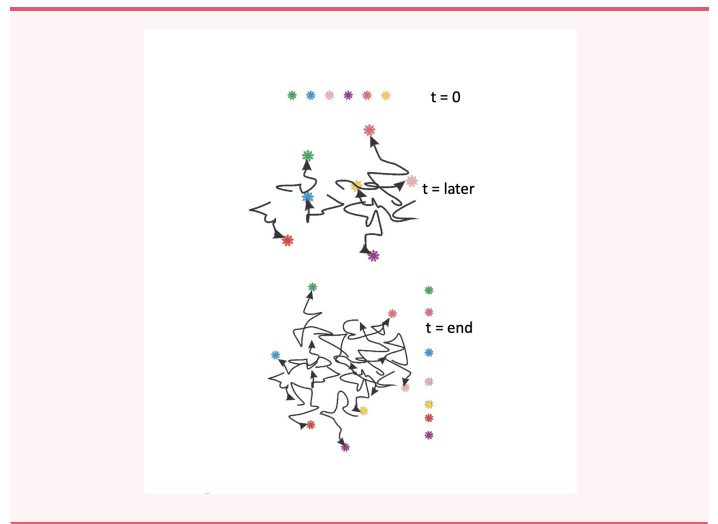
Packing column

- ▶ Reduce longitudinal diffusion = ↓ Plate height (Beneficial)
- ▶ Blocks molecules travel

Equation:

- ▶ $H_{B/U-Term} = (2\gamma D_m)/U$
- ▶ D_m = Diffusion coefficient in MP
- ▶ γ = Obstruction factor
- ▶ 0.6 (packed column)
- ▶ 1.0 (open tubular column)
- ▶ U = MP velocity

B/U-Term Diagram



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C-Term: Resistance to Mass Transfer

C-Term

Ideal chromatography

- ▶ Assumption that analyte can "instantly" equilibrate between 2 phases

MP is always moving the analyte down

Analyte in leading edge of peak are always moving over SP that is deficient in analyte

- ▶ Reverse for trailing edge
- ▶ Out of equilibrium

Equilibrium established when there are analyte at:

- ▶ SP
- ▶ MP
- ▶ Interface

Takes time for analyte to diffuse to/away from phases to match equilibrium constant

- ▶ In SP → Analyte gets further behind than expected
- ▶ In MP → Analyte gets further ahead than expected

Rise to broadening

C_mU-Term: Resistance to Mass Transfer in MP

Space in-between particles depends on particles size/diameter

- ▶ Distance required for diffusion to move analyte
- ▶ Reach interface

Delays in reaching equilibrium depends on distances

C-Term: Resistance to Mass Transfer (cont)

Distance is proportional to size of particle

Equation:

- ▶ $H_{CmU} = (f_m(K')d_p^2 U)/D_m$
- ▶ $f_m(K')$ = Quasi constant → Depends on retention
- ▶ d_p = Particle diameter (units)
- ▶ D_m = Diffusion coefficient of analyte in MP (cm²/s) → 1 cm² = 10⁴mm
- ▶ U = MP velocity

C_sU-Term: Resistance to Mass Transfer in SP

Space in SP depends on SP thickness

- ▶ Distance required for diffusion

Analyte reach MP/SP interface

- ▶ Equilibrium reach
- ▶ Delays depends on distances

Equation:

- ▶ $H_{CsU} = (f_s(K')d_f^2 U)/D_s$
- ▶ d_f = SP thickness
- ▶ D_s = Diffusion Coefficient of analyte in SP

GC:

- ▶ ~0.1-0.5 μm film thickness
- ▶ Controls retention
- ▶ Impact resistance to mass transfer



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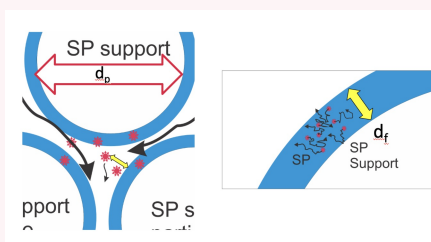
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C-Term: Resistance to Mass Transfer (cont)

LC:

- ▶ Never adjust to thickness → Monolayer
- ▶ Resistance → Negligible
- ▶ Important in MP

CmU and CsU Term Diagram



Resolution

Define 2 peaks of interest (critical pair)

Resolution ▶ Peaks closest together

Resolved

- ▶ Clear separation
- ▶ No analyte mixing
- ▶ Pure peaks

W is not affected by plate height

Successful Separation Isolated peaks

See baseline between peaks

Dependent on resolution

Can use ruler to see if baseline from beginning/end match to baseline between peaks

Quantify Use W

Resolution (R/Rs) ▶ Captures +/- 2σ regions of "Gaussian" peaks

▶ Corresponds to ~ 95.5% of analyte

Resolution (cont)

R improves:

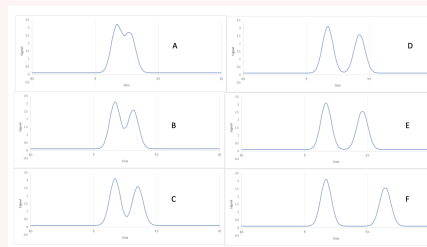
- ▶ Greater Δt_r
- ▶ Smaller W_a and/or W_b
- ▶ Narrow peaks = more baseline exposure

Full W of peak does not matter

- ▶ Only back half (peak 1) and first half (peak 2)

2 neighbouring peaks are resolved when → $R \geq 1.5$

Resolution Diagram

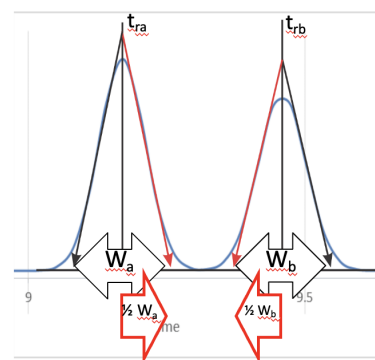


Graph A-C has poor resolution → Overlapping peaks

Graph D has the minimum resolution requirement

Graph E-F has a good resolution → See baseline between peaks

Resolution Equation



$$R = R_s = \frac{\Delta t_r}{\frac{1}{2}W_a + \frac{1}{2}W_b} = \frac{2(t_{rb} - t_{ra})}{W_a + W_b}$$



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Controlling Resolving Power

Control Resolution

Proximity of 2 peaks is important to R
 ▶ Controlled by separation conditions

Quantify proximity:

▶ Selectivity factor → Define as a ratio of distribution constant of 2 peaks

Peaks shares column → Same SP and MP

α = ratio of retention factors

▶ Access from chromatogram
 ▶ Change in selectivity = change in resolution

Effects of Retention and Selectivity on R'

Key variable that controls potential resolutions

▶ Resolving power (R')

R' dependent:

▶ Very sensitive to selectivity (α)
 ▶ Somewhat sensitive to retention (K')
 ▶ Moderately sensitive to efficiency of column (N)
 ▶ Choice of column
 ▶ Choice of MP (LC only)

α control by:

▶ Differential interactions between:
 Analyte ↔ MP ↔ SP

Controlling Resolving Power (cont)

N control by:

▶ Column (L)
 ▶ VD equations
 ▶ Type of column
 ▶ SP thickness
 ▶ Operating conditions

K' control by:

▶ SP type
 ▶ Phase Ratio (SP thickness)
 ▶ MP type (LC only)

Effects of R' on Retention Time

↑ R' = ↑ Total run time

▶ Interplay

Interplay between R' and t_r as a function of K'

R':

▶ N and α ~ constant when K' is alter
 ▶ Replace terms with Q

t_{rb} :

▶ N, H, α , U ~ constant
 ▶ Assume R' is not changing dramatically
 ▶ Replace constant terms with Q

Effects on Retention and Selectivity R'

$$K = \frac{C_s}{C_m} \quad k'_a = K_a \frac{V_s}{V_m} \quad k'_b = K_b \frac{V_s}{V_m}$$

$$\alpha = \frac{k'_b}{k'_a} \quad \alpha = \frac{k'_b}{k'_a} = \frac{t_{r_b} - t_m}{t_{r_a} - t_m}$$

$$R' \approx \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k'_b}{1 + k'_b} \right) \frac{\sqrt{N}}{4} \iff N \approx 16R'^2 \left(\frac{\alpha}{\alpha - 1} \right)^2 \left(\frac{1 + k'_b}{k'_b} \right)^2$$

$$\text{IF } k'_a \approx k'_b = k' \quad \text{THEN } \alpha = \frac{k'_b}{k'_a} \approx 1$$

$$R' \approx (\alpha - 1) \left(\frac{k'}{1 + k'} \right) \frac{\sqrt{N}}{4} \iff N \approx 16R'^2 \left(\frac{1}{\alpha - 1} \right)^2 \left(\frac{1 + k'}{k'} \right)^2$$



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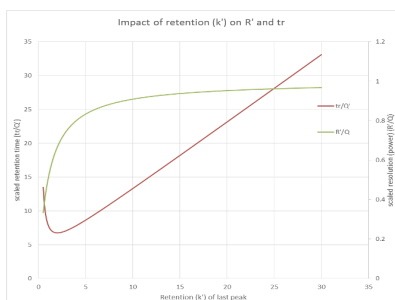
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Effects of R' on tr



$$R' \approx \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k'_b}{1 + k'_b} \right) \frac{\sqrt{N}}{4} \longrightarrow \frac{R'}{Q} \approx \left(\frac{k'_b}{1 + k'_b} \right)$$

$$tr_b \approx \frac{16R^2H}{U} \left(\frac{\alpha}{\alpha - 1} \right)^2 \frac{(1 + k'_b)^3}{(k'_b)^2} \longrightarrow \frac{tr_b}{Q'} \approx \frac{(1 + k'_b)^3}{(k'_b)^2}$$

Notice that R' increases significantly at low K' but plateaus at large K'

- Don't use separations with small K' (low R')

Notice that tr increases linearly with increasing k' BUT R' plateaus at large k'

- Therefore there is no real benefit to sep'ns with large k's (b/c R' ≈ constant)



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