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 = ↑ [Analye]
	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

Separation Process

Occurs in tube/plate (TLC)

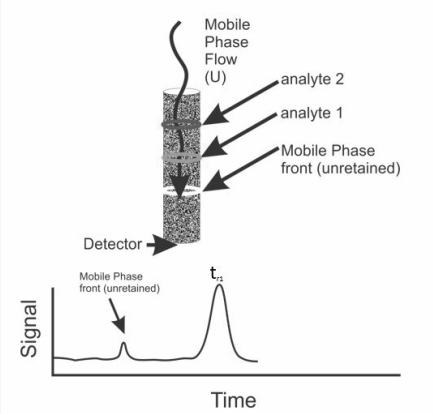
Drive MP through column
- consistent velocity
- Use a pump (LC) → HPLC
- Use capillary action (TLC) → Dip plate in MP
- Use gas pressure (GC) → Store MP in HP-cylinder + attach to gas regulator

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

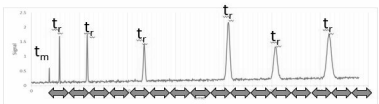
Measure Retention (K')	Variables ▸ L=Length of column ▸ U= MP velocity ▸ V = Analyte velocity ▸ t_m = retention time of MP ▸ t_r=retention time of retained species ▸ K=distribution constant ▸ C_s= [Analyte] in SP ▸ C_m= [Analyte] in MP
	t_r ➔ use to identify analyte
	Simple matrix ➔ 1< K' <10
	Complex matrix ➔ 0.5< K' <20
	K' ▸ Determined by chromatogram ▸ Controlled by equillibrium ▸ 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

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

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

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



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= Lenght of column ▸ W = peak width at baseline ▸ σ = Standard deviation (unit of lenght)
	Desirable ▸ ↑ N = ↓ H = ↓ σ

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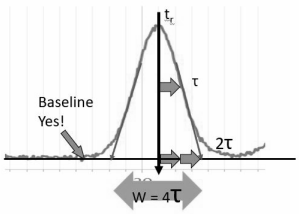
Efficiency (cont)

	W range = -2τ tp + 2τ
	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
	$W_{1/2} \neq 1/2\ W$

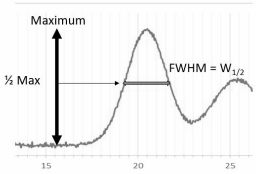
"Gaussian Peak" At W

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

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



Full-Width at Half Maximum



$$N = 5.54\left(\frac{t_r}{W_{1/2}}\right)^2$$

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 Deemeter Graph	Produce the overall curve with distinct minimum ▸ Corresponding to N_{max} and fixed L

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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)

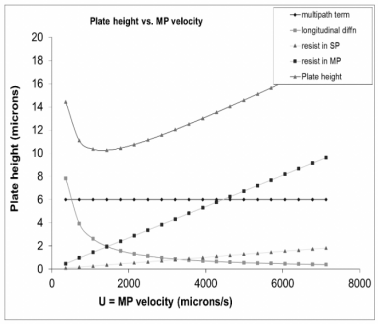
C_sU-Term:

- Linear increasing (blue line)

C_mU-Term:

- Linear increasing (yellow line)

Van Deemter Graph (copy)



$$H = A + \frac{B}{U} + (Cs + Cm)U$$

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

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

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

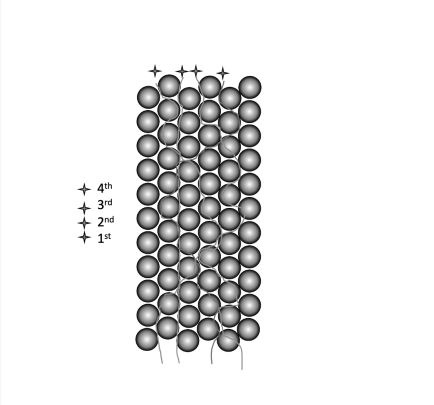
- Crack, voids, etc

A-Term: Multipath Band Broadening (cont)

Equation:

- $H_{A\text{-}term}$= 2λd_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 ↓)

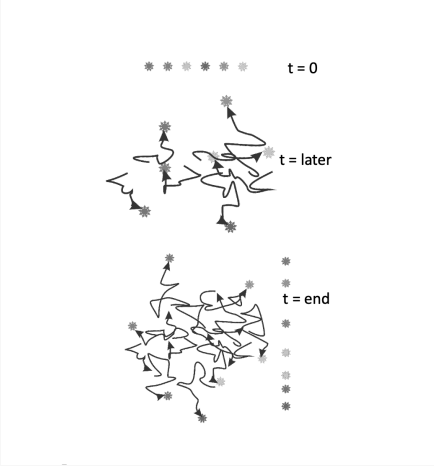
Packing column

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

Equation:

- $H_{B/U\text{-}Term}$ = (2γD_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 analyre 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 (cm^2/s) → 1 cm^2= 10^4mm ▸ 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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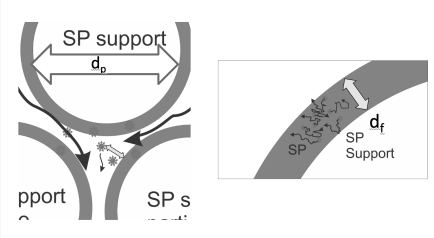
C-Term: Resistance to Mass Transfer (cont)

LC:

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

CmU and CsU Term Diagram



Resolution

Define Resolution	2 peaks of interest (critical pair) • 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 Resolution (R/Rs)	Use W • Captures +/- 2σ regions of "Gaussian" peaks • Corresponds to ~ 95.5% of analyte

Resolution (cont)

R improves:

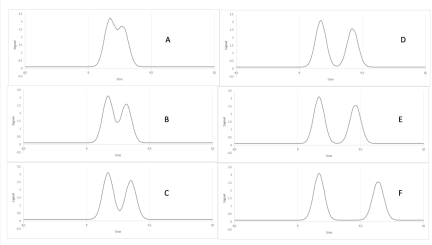
- Greater Δt_r
- Smaller W_a and/or W_b
- Narrow peaks = more baseline expose

Full W of peak does not matter

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

2 neighbouring peaks are resolved when → R ≥1.5

Resolution Diagram

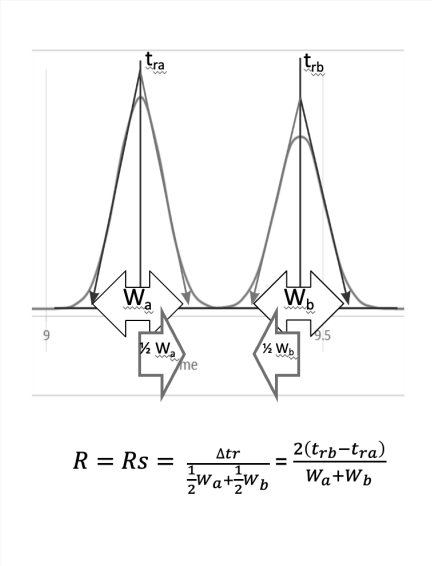


Graph A-C has poor resolution → Overlapping peaks

Graph D has the minimum resolution requirement

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

Resolution Equation



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

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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 tr as a function of K'
	R': ▸ N and α ~ constant when K' is alter ▸ Replace terms with Q
	trb: ▸ N,H,α, U ~ constant ▸ Assume R' is not changing dramatically ▸ Replace constant terms with Q

Effects on Retention and Selectivity R'

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

$$\alpha = \frac{K_b}{K_a} \qquad \alpha = \frac{k'_b}{k'_a} = \frac{tr_b - t_m}{tr_a - t_m}$$

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

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

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

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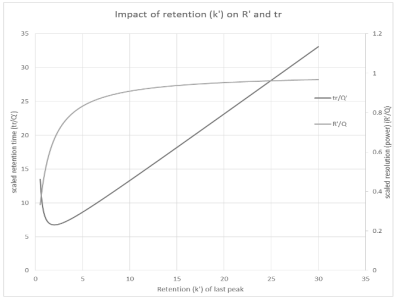
Effects of R' on tr



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

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

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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