Prince of Songkla University

Separation Methods

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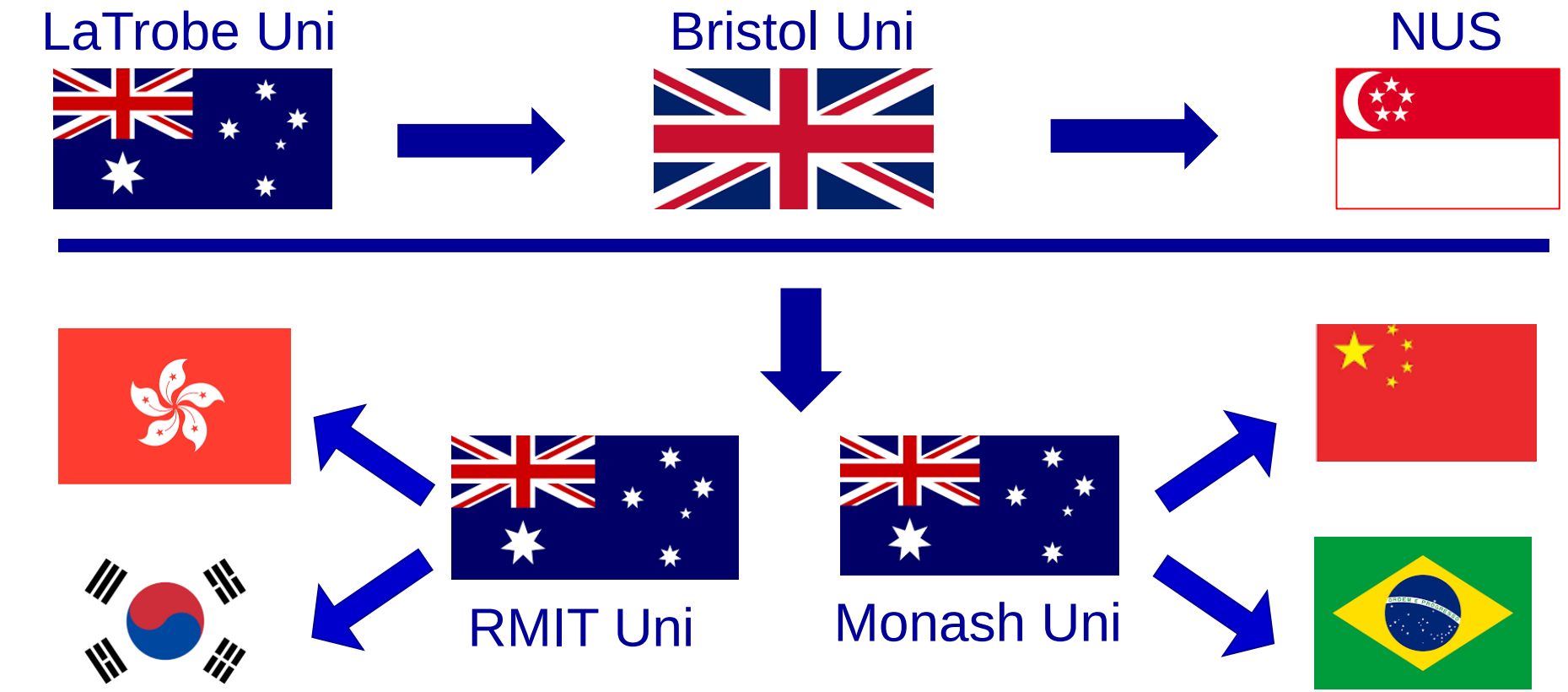
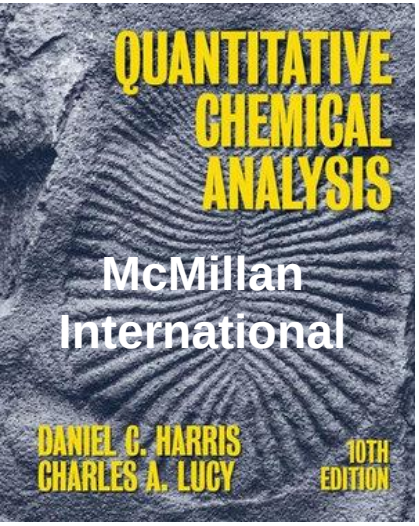




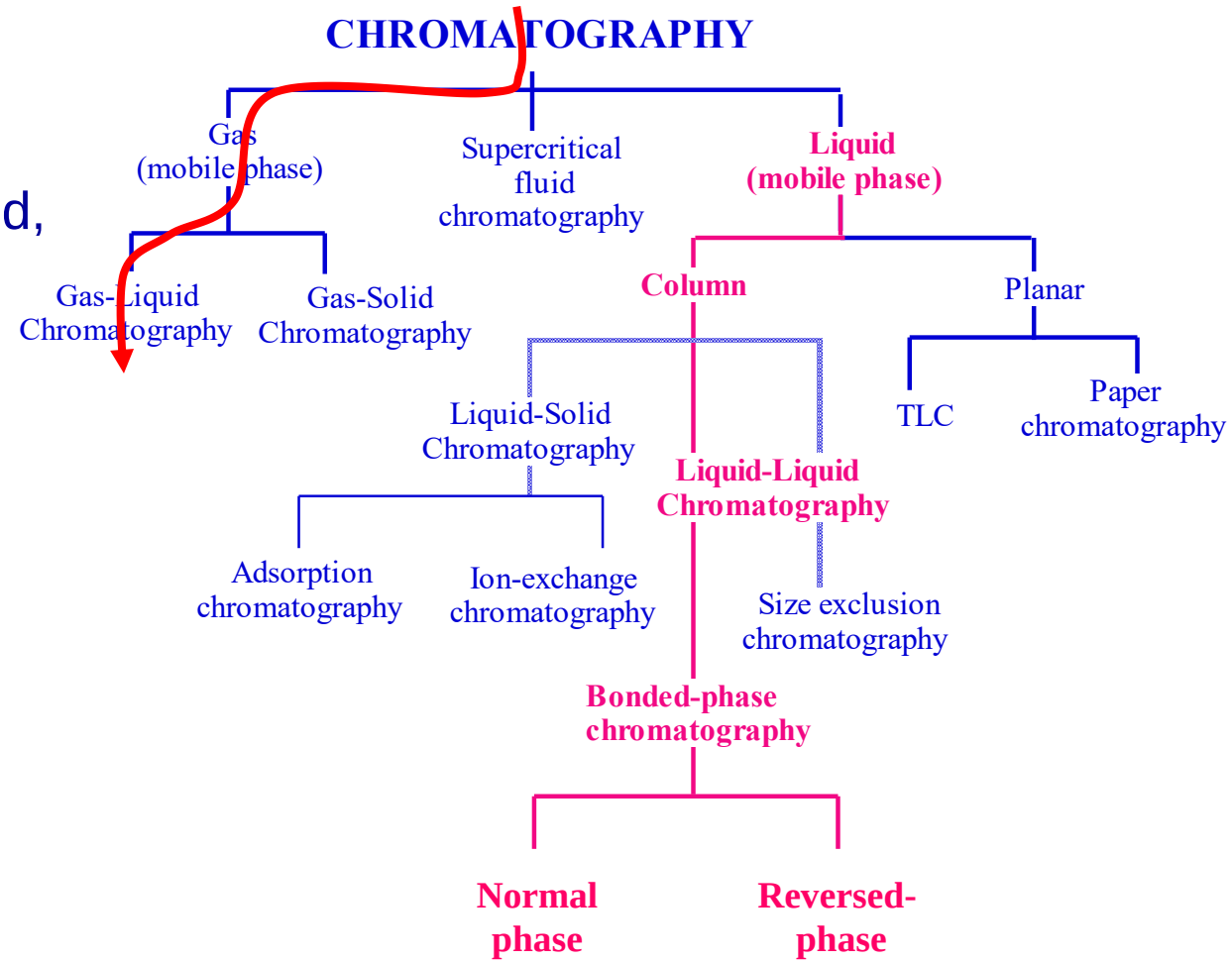
Classification of Separation Methods

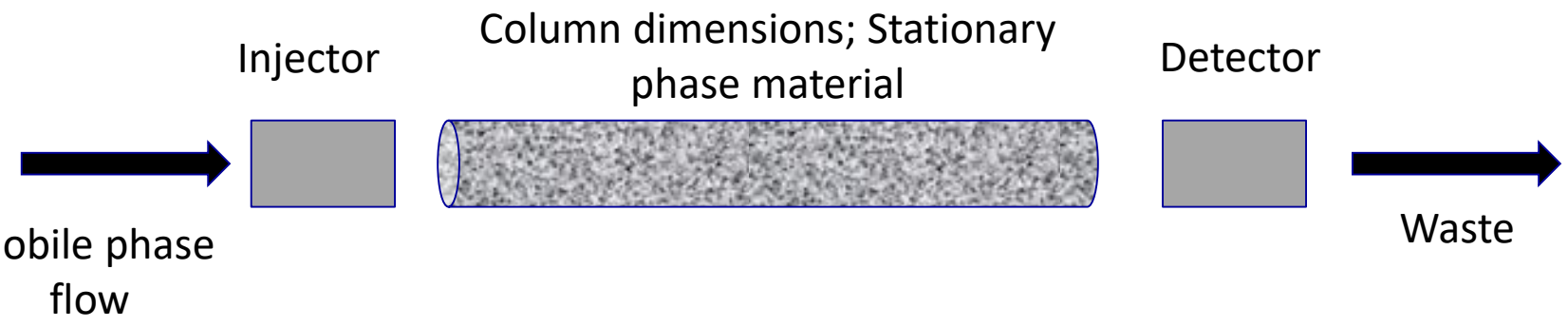
Mobile Phase: gas, liquid, supercritical fluid

Stationary Phase: Solid, liquid, bonded phase, nature

Architecture:

Column (packed, open tube), planar





Non-polar / polar organic phase eluent	Non-polar / polar bonded polymer or solid support; packed	**HPLC**
Supercritical fluid & organic modified eluent	Non-polar / polar bonded polymer support; GC or HPLC-like; packed or capillary	**SFC**
Electrolyte, e.g. carbonate	Ion-exchange sites on polymer support; packed	**IC**
Gas	Solid support e.g. catalyst or sorbent, capillary or packed	**GSC**
	Polar or non-polar polymer; Packed, or on capillary column walls	**GLC; GC**

Separation Methods
Course Coverage - Focus on GC

- **Some theoretical development**
  Diffusion; van Deemter eqn; Golay Eqn; Maximum performance; K=kβ; non-linear effects; Fast GC & method translation
- **Gas Chromatography technology**
  Capillary GC columns; GC Phases; Retention indices; Injection; Detection; Derivatisation; Sampling;
- **Comprehensive two-dimensional gas chromatography & multidimensional gas chromatography**
  ”Tutorial”; Phase choices; Higher separation power; Sensitivity; Modulation; 2D methods; Presentation; Structure;
- **Analytical mass spectrometry**
  Ionisation; Example data extraction GC-MS; Library searching; Collisional activation, MS/MS; Target/untargeted analysis; Derivatisation; Internal standards; Portable GC-MS.

Describing Elution Behaviour

- Partition chromatography (incl. most GC and HPLC)

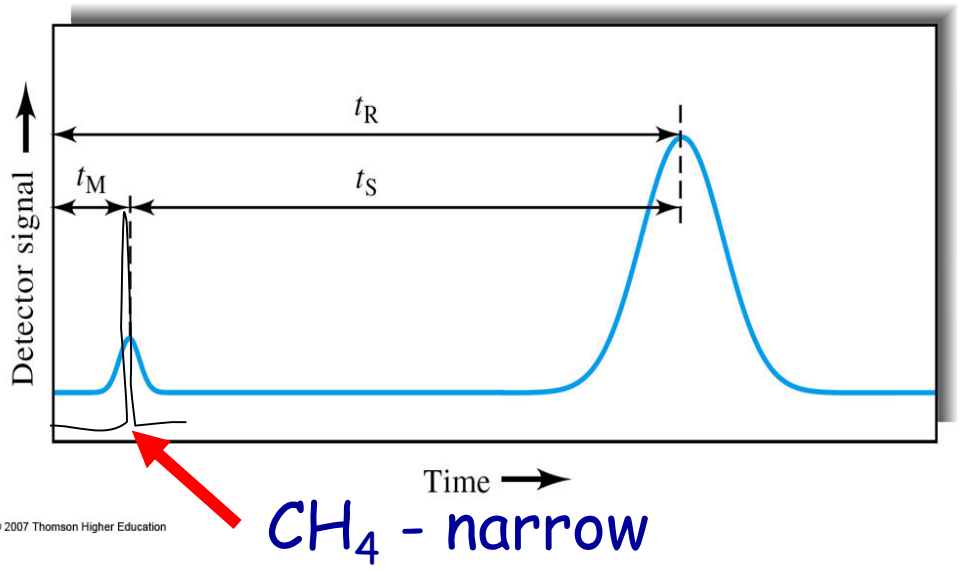
Retention time, t_R, **Distribution** (Partition) coefficient, K, and **retention** (or capacity) factor, **k** (*** not k' ***)

- t_M = retention time (min (or vol or distance)) for unretained material = void or dead volume *(injection peak ~ methane for FID)*
- t_R = retention time of analyte (min (or vol or distance))
- Adjusted retention time:

$$t'_R = t_R - t_M$$

$$(t'_R = t_S)$$

(t_S, time spent in stationary phase-Skoog)



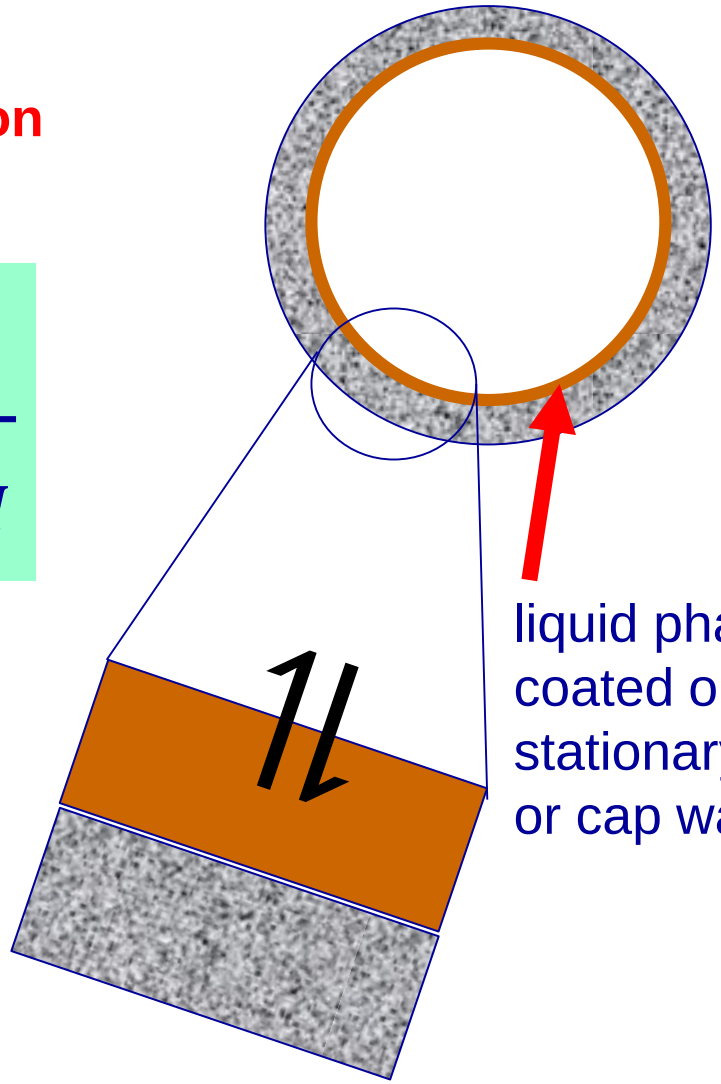
** Check the document: **Nomenclature in Chromatography**

Describing Elution Behaviour

The tendency for analyte to be "dissolved" in the stationary phase or on sorbent is given by the **Distribution** (partition) **coefficient**:

$$K = \frac{C_S}{C_M} = \frac{n_S/V_S}{n_M/V_M}$$

where C_S and C_M are the analyte concentrations in the stationary and mobile phases resp.



Describing Elution Behaviour

For analyte A, we define the **retention factor**, k_A as:

Thus

$$k_A = K_A \frac{V_S}{V_M} \longrightarrow v = u \times \frac{1}{1+k_A}$$

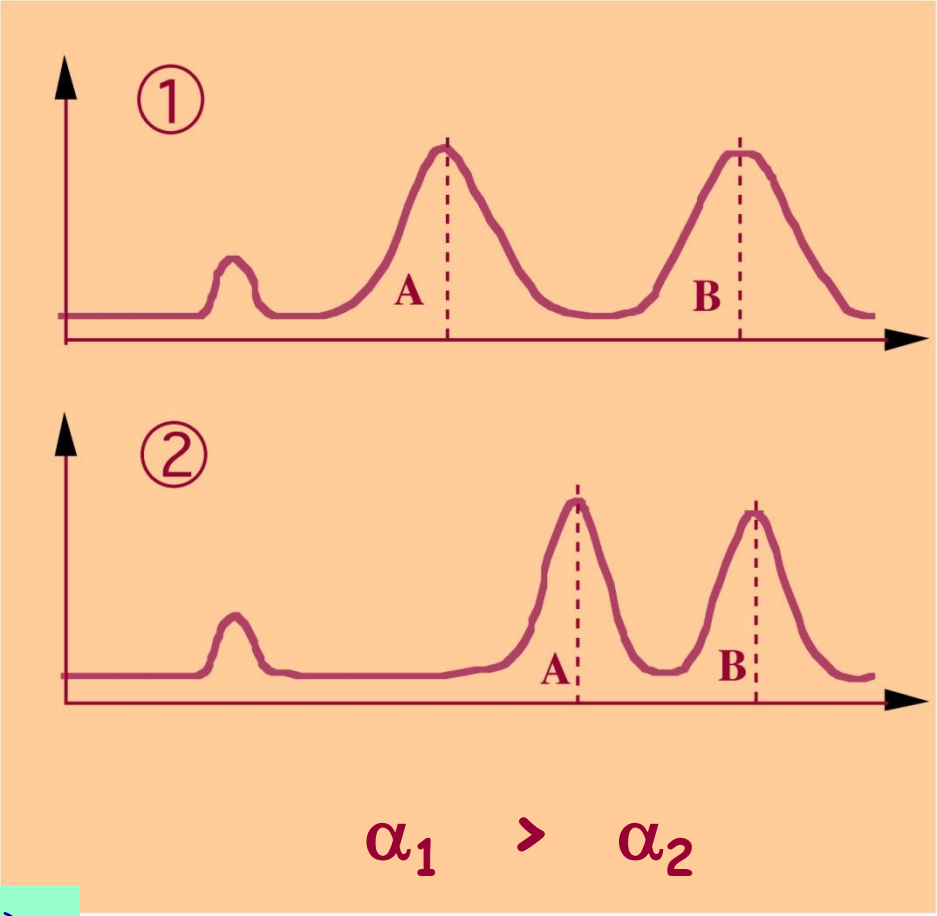
$$\frac{L}{t_R} = \frac{L}{t_M} \times \frac{1}{1+k_A} \longrightarrow k = \frac{(t_R - t_M)}{t_M} = \frac{t'_R}{t_M}$$

- k measures the extent of retention of an analyte.
- "factors out" variables like column length and flow rate.....allows comparison.
- k should ideally be in range **2-5**, but in practice **1-15** is acceptable. **(for isothermal / isocratic analysis) ** for a simple sample**

Describing Elution Behaviour

Selectivity, α

- How well does a column separate (**or retain??**) two analytes?
- For analytes A and B, where $t_{RA} < t_{RB}$:



$$\alpha = \frac{K_B}{K_A} = \frac{k_B}{k_A} = \frac{(t_R)_B - t_M}{(t_R)_A - t_M} = \frac{(t'_R)_B}{(t'_R)_A}$$

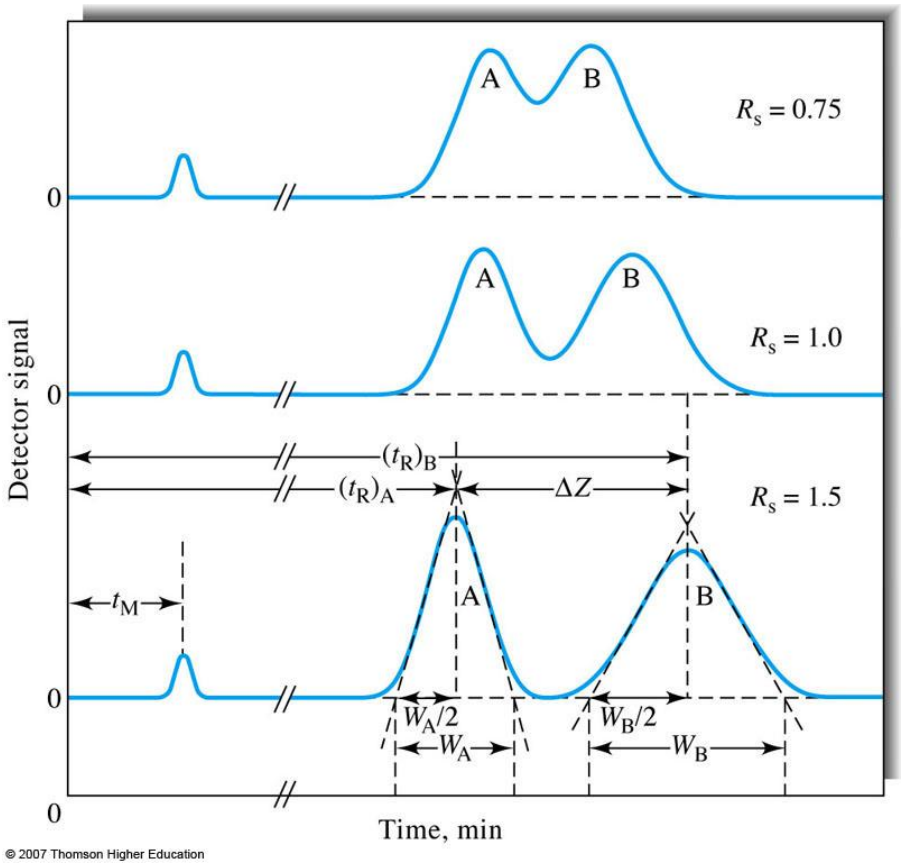
Describing Elution Behaviour

Band Broadening and Resolution

- α does not allow for (does not consider) band broadening, so use Resolution, **Rs**, as a better measure of peak separation.

$$R = \frac{\Delta Z}{\frac{W_A}{2} + \frac{W_B}{2}} = \frac{2\left[\left(t_R\right)_B - \left(t_R\right)_A\right]}{W_A + W_B}$$

- For baseline resolution, Rs ≥ 1.5 •



$$Rs \sim \Delta t_R / w_A$$

Band Broadening: Plate and Rate Theory

Diffusion

- Diffusion is **one cause** of band broadening.
- Spontaneous spreading of analyte from region of high concentration to region of low concentration.
- Described by the ***flux, J,*** *(mol/m^2/s) (**Fick's law**) where D = Diffusion coefficient (**Einstein-Stokes**) (m^2/s)*

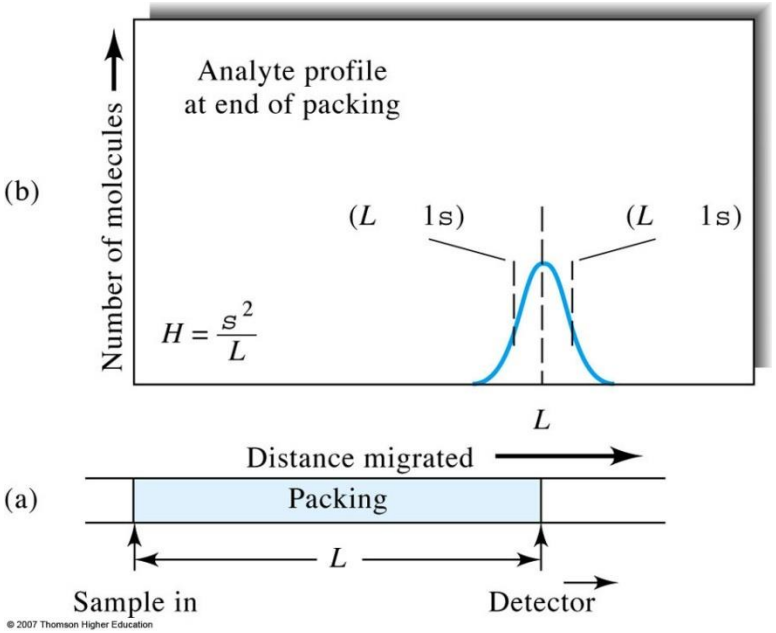
$$J = -D\frac{dc}{dx}$$

- Where x is distance and c is [ ]. **In liquids, the value of D for most** analytes is approx 10^{-9} m^2/s (~10^{-5} cm^2/s). In gases, diffusion is much faster.

Time

Band Broadening-Plate and Rate Theory

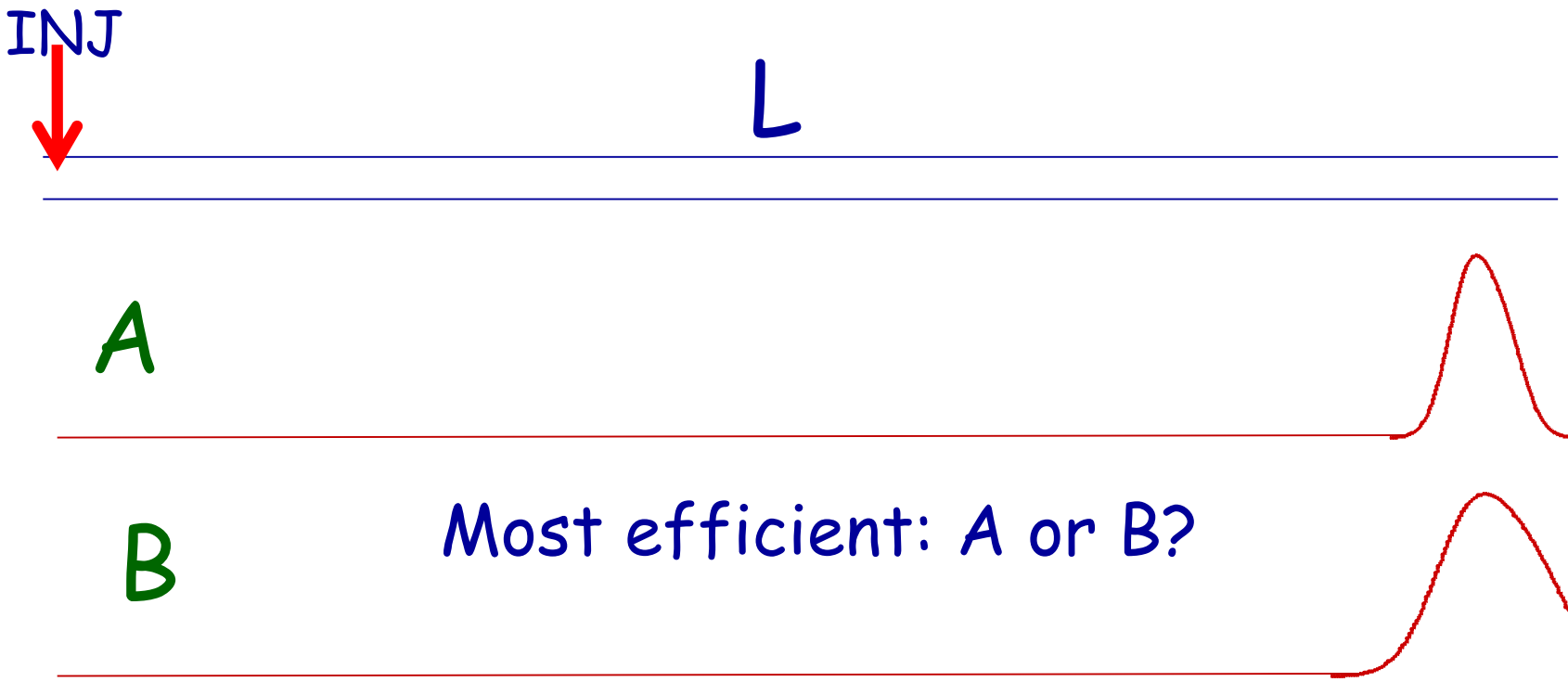
- ***amount of band broadening*** RELATED TO ***distance it travels through the column***.
  Smaller plate height, H, = less the band broadening.✓✓ **column efficiency.**
- N is determined experimentally from the t_R, and the peak width (or peak width, half max):
- For a column of length, L, the number of theoretical plates, N, is:



$$N = \frac{L}{H} = 16\frac{t_R^2}{w_b^2} = 5.54\frac{t_R^2}{w_{0.5}^2}$$

Which is best - a large or small value of H?

Small H →narrow peak →more 'plates' →best Rs



Measure of efficiency; N:

= distance travelled / peak spread (**how far/how wide**)

= L / width parameter (σ)

OR = L^2 / variance (σ^2)

So … **$N = L^2 / \sigma^2$** IN volume, time **OR** distance units

Band Broadening

Band Broadening Processes-Generalised Equation

- Band broadening: described by the more general equation:

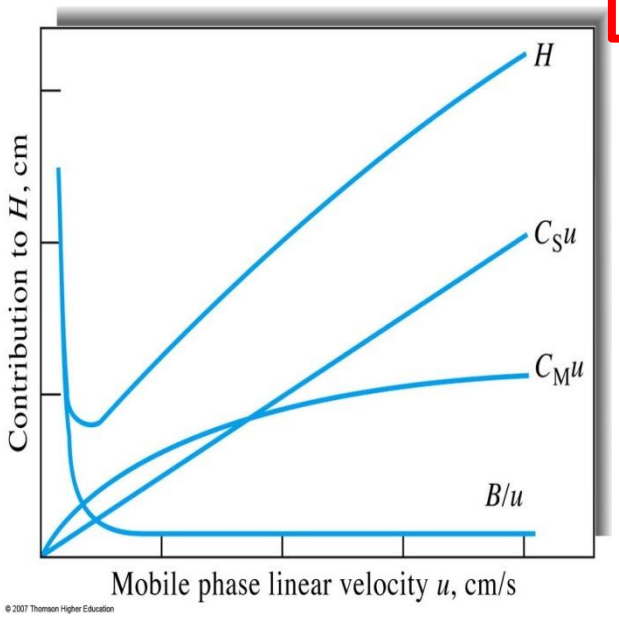
$$H = A + \frac{B}{\bar{u}} + C_S\bar{u} + C_M\bar{u}$$

Unequal flow paths, "Eddy diffusion"

Stationary phase mass transfer

Longitudinal diffusion

Mobile phase mass transfer



Where ū is the linear flow rate, and A, B, C_S and C_M are constants (defined below) •

Band Broadening

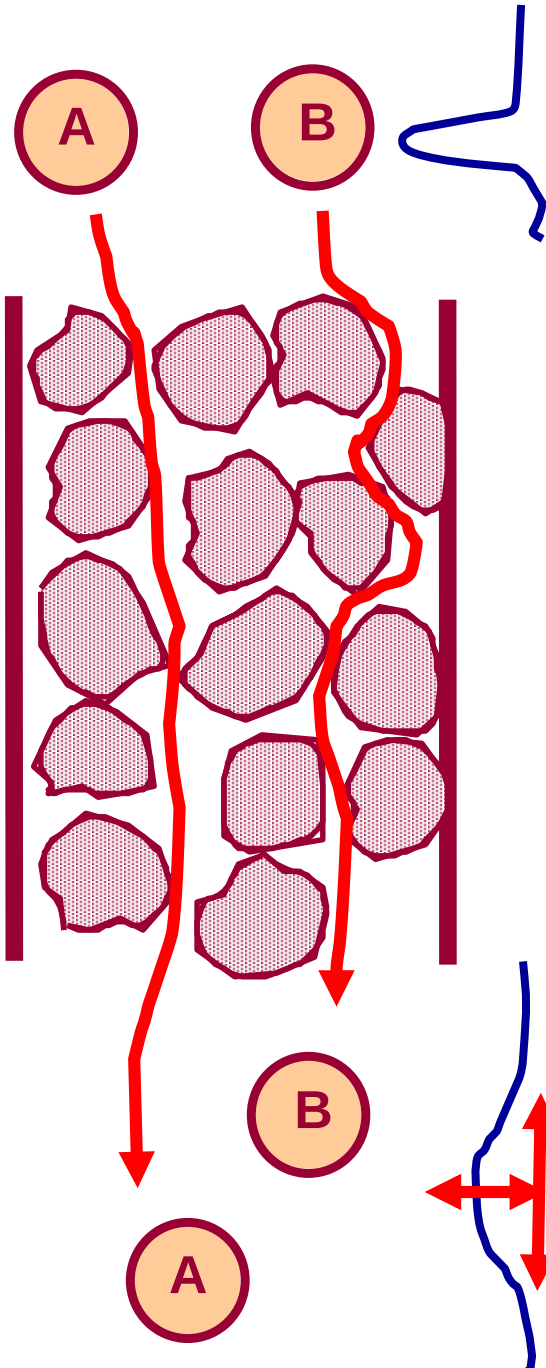
Multipath (Eddy diffusion term), *A*

"Eddy Diffusion" describes band broadening due to unequal paths travelled by analyte through column.

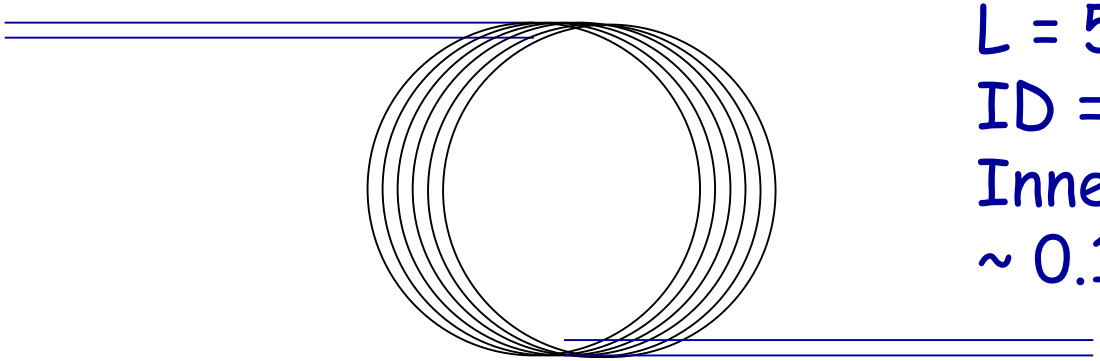
$$A = 2\lambda d_p$$

- ***d_p = packing particle diameter,***
- ***λ= constant related to nature of packing.***

- i.e. the smaller the particles, the smaller the unequal paths term **so they are closer to the averaged value**.



Multipath (Eddy diffusion term), A



L = 5 – 200 m
ID = 0.1-0.53 mm
Inner phase coating df
~ 0.1-2.0 μm

CAPILLARY COLUMN;
no particle packing; no 'd_p' term, so A = 0

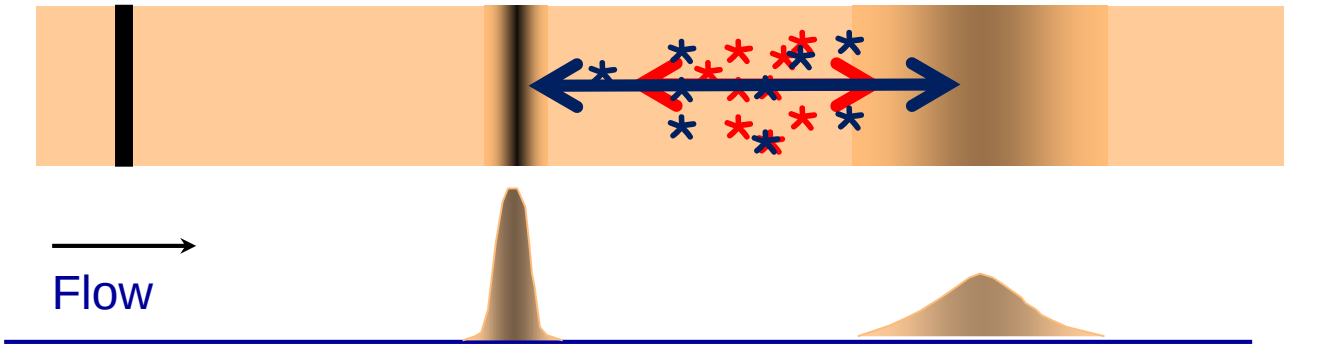
Band Broadening

Longitudinal Diffusion, B/ū

Spreading of the analyte along the column, by diffusion in the mobile phase. Given by:

$$\frac{B}{\bar{u}} = \frac{2\gamma D_M}{\bar{u}}$$

- Where D_M is the diffusion coefficient of the analyte in the mobile phase, and is a const.

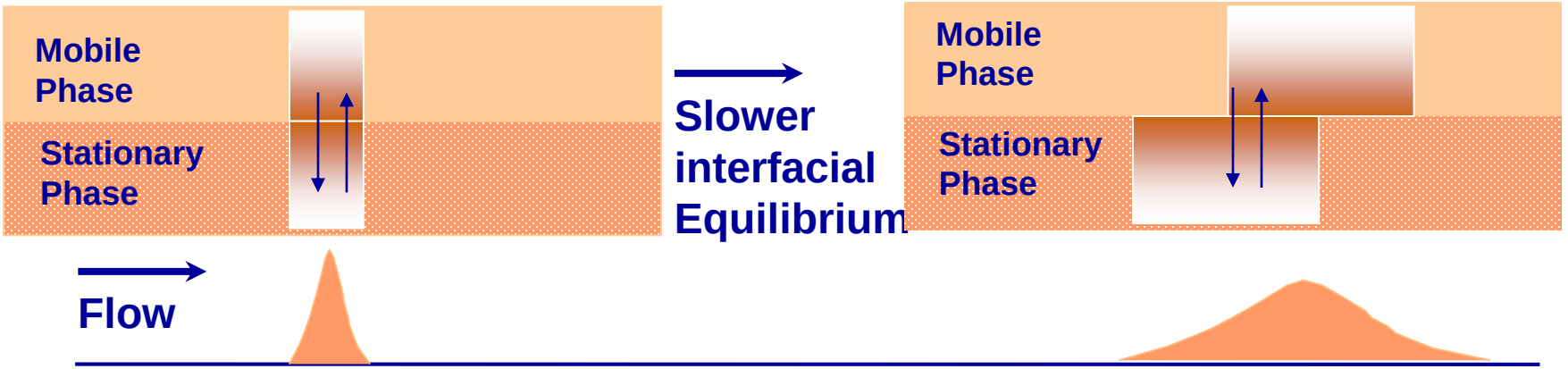


Band Broadening

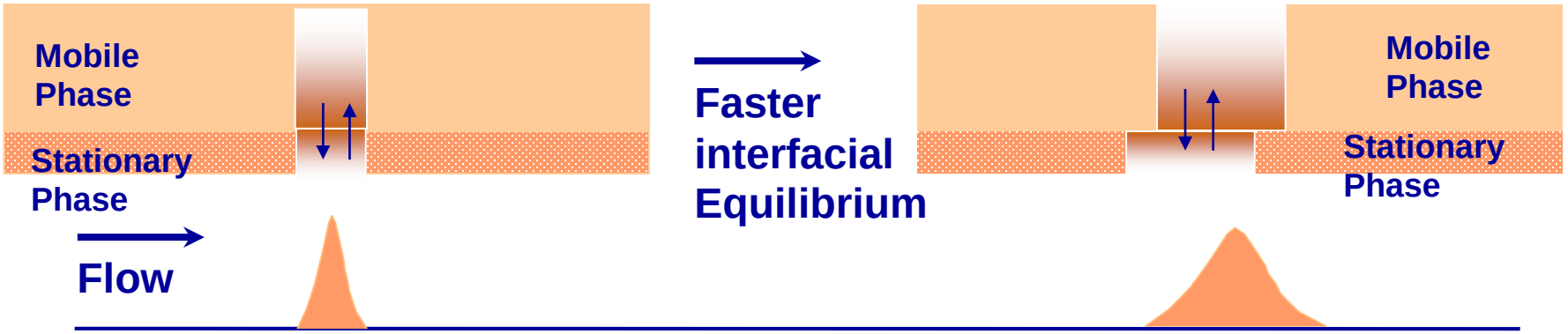
Don't confuse Cs for Cs!!

Stationary phase mass transfer term, C_s ū

- Related to the Rate of equilibration of analyte between the stationary/mobile phases. (Diffusion process…)
- Depends on the immobilised liquid thickness of stationary phase.
- Thicker films mean that molecules need to travel further within stationary phase before emerging into the mobile phase and being eluted.



Band Broadening



For liquid stationary phases, e.g. HPLC and GC

$$C_S \bar{u} = \frac{f(k)\, d_f^2}{D_S}\, \bar{u}$$

(Note Cs term here)

- Where:
  - d_f = film thickness of adsorbed liquid of stationary phase,
  - D_S = diffusion coefficient of analyte in stationary phase
  - **SO thin films favour small C_s; small u always favours small Cs**

Band Broadening

Mobile phase mass transfer term, C_M ū

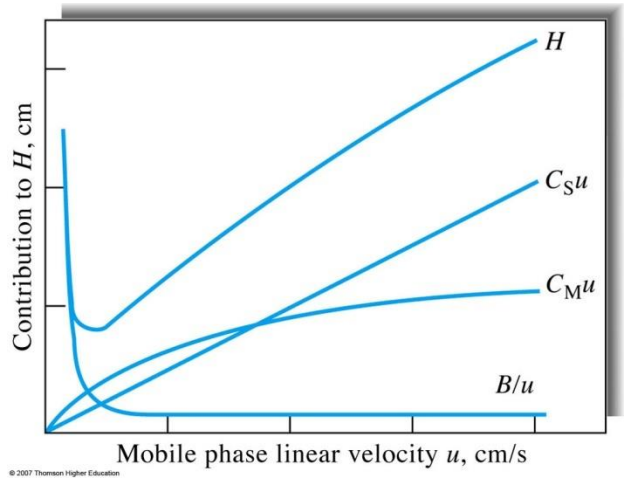
- C_M depends on analyte diffusion coefficient D_M.
- For packed columns, C_M is proportional to the square of particle diameter, d_p; for capillary columns, replace d_p by capillary I.D. d_c.

PACKED

$$C_M \bar{u} = \frac{f'(k) d_p^2}{D_M} \bar{u}$$

CAPILLARY

$$C_M \bar{u} = \frac{f(k)\, d_c^2}{D_M}\, \bar{u}$$



$$f(k) = \frac{1+6k+11k^2}{96\,(1+k)^2}$$

Wide bore cap GC columns less efficient; d_c larger = C_m larger.

D_M for N_2 is smaller, so C_M is larger for N_2; less efficient. WHY?

(how quickly does solute get to the stationary phase?)

B Term: D_M is in the NUMERATOR
C Term: D_M is in the DENOMINATOR
What does this mean?

Higher D_M is BAD for B Term, but is GOOD for C_M Term!!

THEY have the OPPOSITE Effects!

Why??

1. More diffusive carrier gas leads to spreading in mobile phase;
2. More diffusive carrier gas leads to faster travel to the stationary phase interface – this is needed for separation! Many equilibration steps…
∴ OPPOSITE

Band Broadening

Band Broadening Processes-Van Deemter Equation

- Band broadening in GC described by the equation:

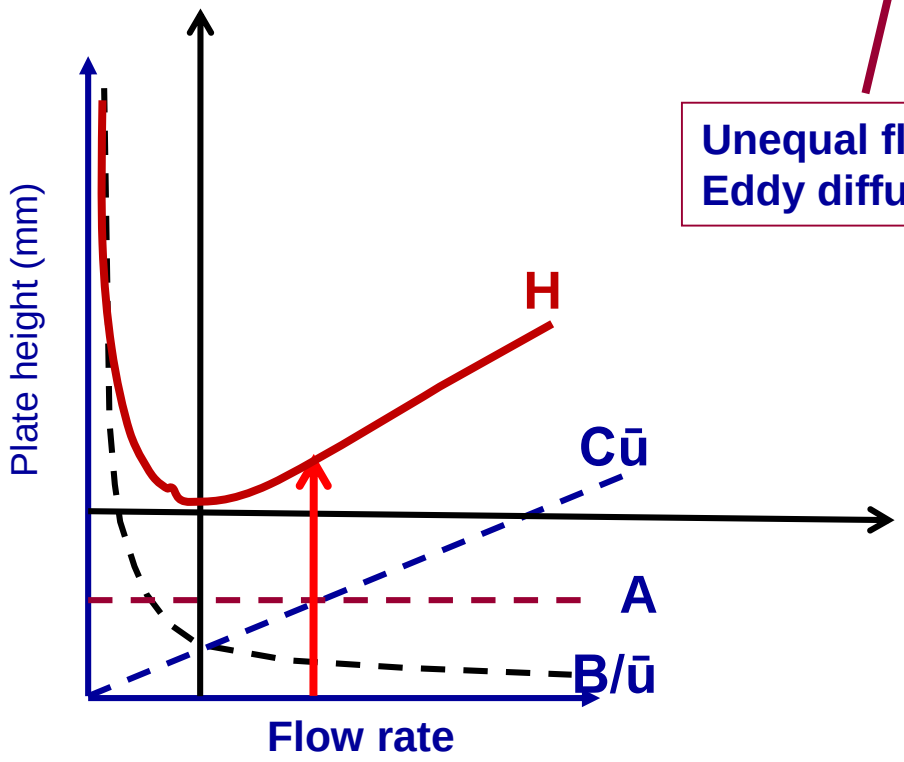
$$\bar{u}_{opt} = \sqrt{B/C}$$

$$H = A + \frac{B}{\bar{u}} + C\bar{u}$$

Unequal flow paths, Eddy diffusion

Longitudinal diffusion

Non-equilibrium



$$H_{min} = A + 2\sqrt{BC}$$

OPGV ~ 2 x $\bar{u}_{opt}$

Band Broadening

Band Broadening Processes-Golay Equation for capillary columns

- Band broadening equation for capillary column GC - the eddy diffusion term -> 0 ($A \propto d_p$; $d_p = 0$), and thus:

$$H = \frac{B}{\overline{u}} + C\overline{u}$$

Who is this 'Golay' guy?

For capillary cols, we usually use a best temperature program rate of ~ 10 / unretained peak time in min. t_M = 0.5 min; use a heating rate of 20 °C/min (see Hinshaw: GC in the FAST Lane paper)

5. Band Broadening

5.3.4 Calculate the maximum (theory) efficiency for a capillary column…. ONLY Need d_c and k values

$$H_{min} = A + 2\sqrt{BC} \longrightarrow H_{min} = 2\sqrt{BC}$$

$$B = 2\gamma D_M = 2 D_M \qquad C = f(k)\, d_c^2 / D_M \qquad f(k) = \frac{1+6k+11k^2}{96\,(1+k)^2}$$

$$H_{min} = 2\sqrt{BC}$$

$$= 2\,(2 D_M \,.\, f(k)\, d_c^2 / D_M)^{0.5} = 2\, d_c\, (1/48\, f(k))^{0.5}$$

Allow k = 4
d_c = 0.25 mm

$$= 2/7\, d_c\, (f(k))^{0.5} = 2 \times 2.8 / 7\, d_c \sim 0.8\, d_c = 0.2 \text{ mm}$$

SO 0.25 mm ID col 25 m long for a k = 4 solute Has 25000mm/0.2mm = 125,0000 plates

Now: an EXERCISE

Calculate the efficiency for the UNRETAINED PEAK (eg Methane)

CH_4 in capillary column; k = …….

$$f(k) = \frac{1+6k+11k^2}{96\,(1+k)^2}$$

$H_{min} = 2/7\ d_c\ (f(k))^{0.5} \sim dc / 4$ (See Last Slide)

So methane should give us the narrowest peak

SO 0.25 mm ID col 25 m long for a k = 0 solute

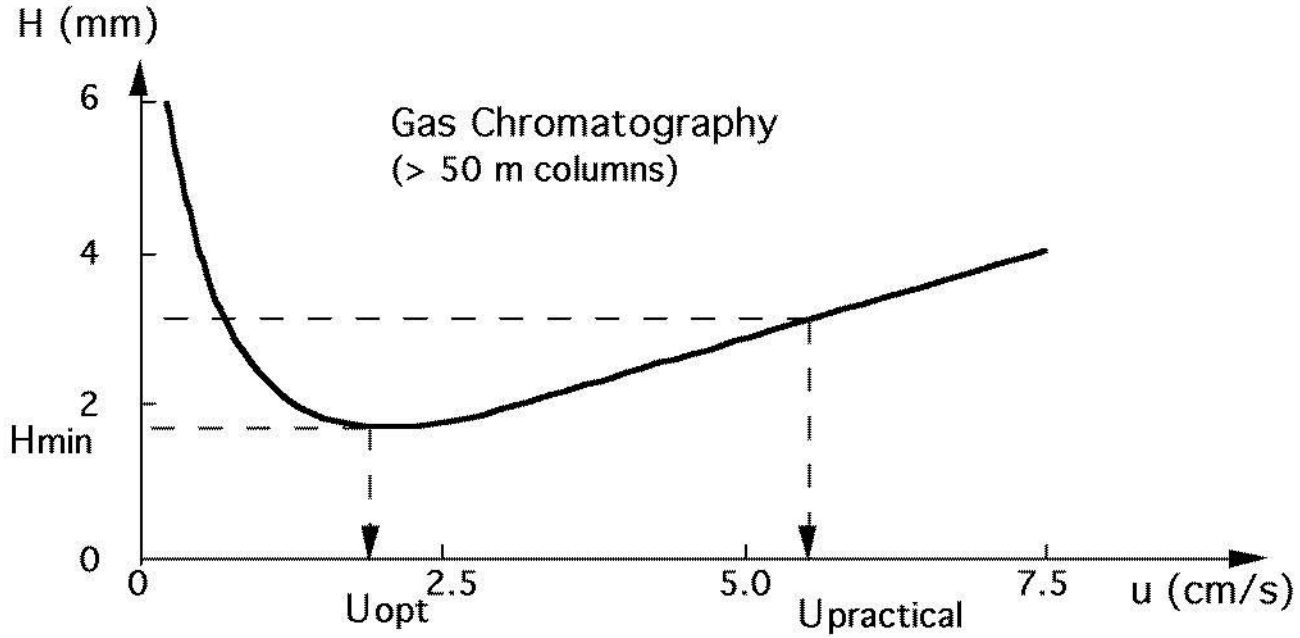
H_{min} = 0.25 / 4 ~ 0.06 mm

The column has 25000mm/0.06 mm ~ 400,000 plates

Optimisation of Separations

Reducing band broadening

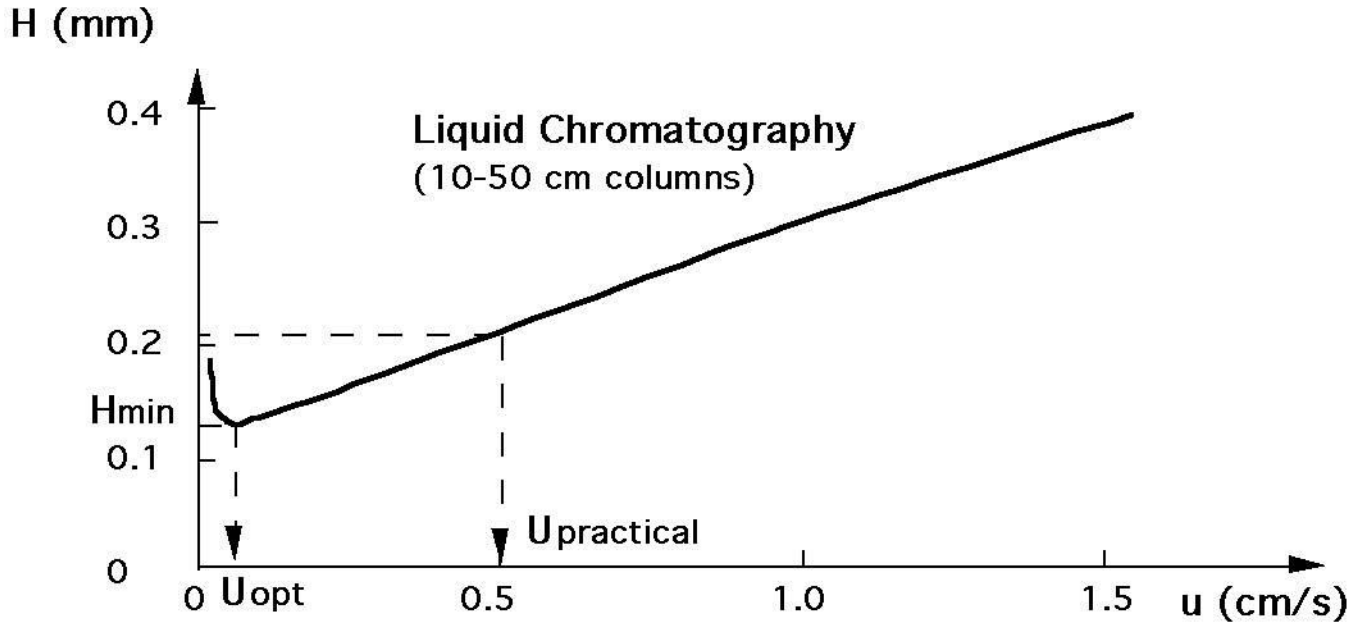
- Choose optimum ū (or near optimum).
- GC- reduce B/ū term by decreasing Temp, and reducing D_M (for B term) using narrow bore columns (won't work in LC, as D_M already small).



Optimisation of Separations

- LC -reduce C_s (non-equilib term) by minimising coating thickness on particles (d_f). **(NOTE: NEW CORE SHELL PHASES)**

$$C_s u \propto d_f$$



- Both GC and LC - minimize A (eddy diffusion term) by reducing packing particle diameter (d_p). **(or capillary columns & narrow ID)**

Optimisation of Separations

Relationship between Resolution and Column Properties

- For two analytes with similar k values,

$$R_S = \frac{(t_R)_B - (t_R)_A}{W} = \frac{(t_R)_B - (t_R)_A}{(t_R)_B} \times \frac{\sqrt{N}}{4}$$

- $$R_S = \frac{k_B - k_A}{1 + k_B} \times \frac{\sqrt{N}}{4}$$
- Rearrangement permits calculation of the number of plates required to give a certain resolution: Practice this for a few example settings…

$$N = 16 R_S^{\,2} \left(\frac{1 + k_B}{k_B - k_A} \right)^2$$

Optimisation of Separations

Methods for improving Resolution

1. **Based on column construction:**

   (a) column length increased ***(increase N).***

   (b) more uniform packing, smaller particles ***(decrease A, C_M term).***

   (c) narrower bore column (capillary) ***(increase N).***

   (d) different stationary phase ***(change K, k).*** **FOR COMPLEX SAMPLES?**

2. **Based on column operation**

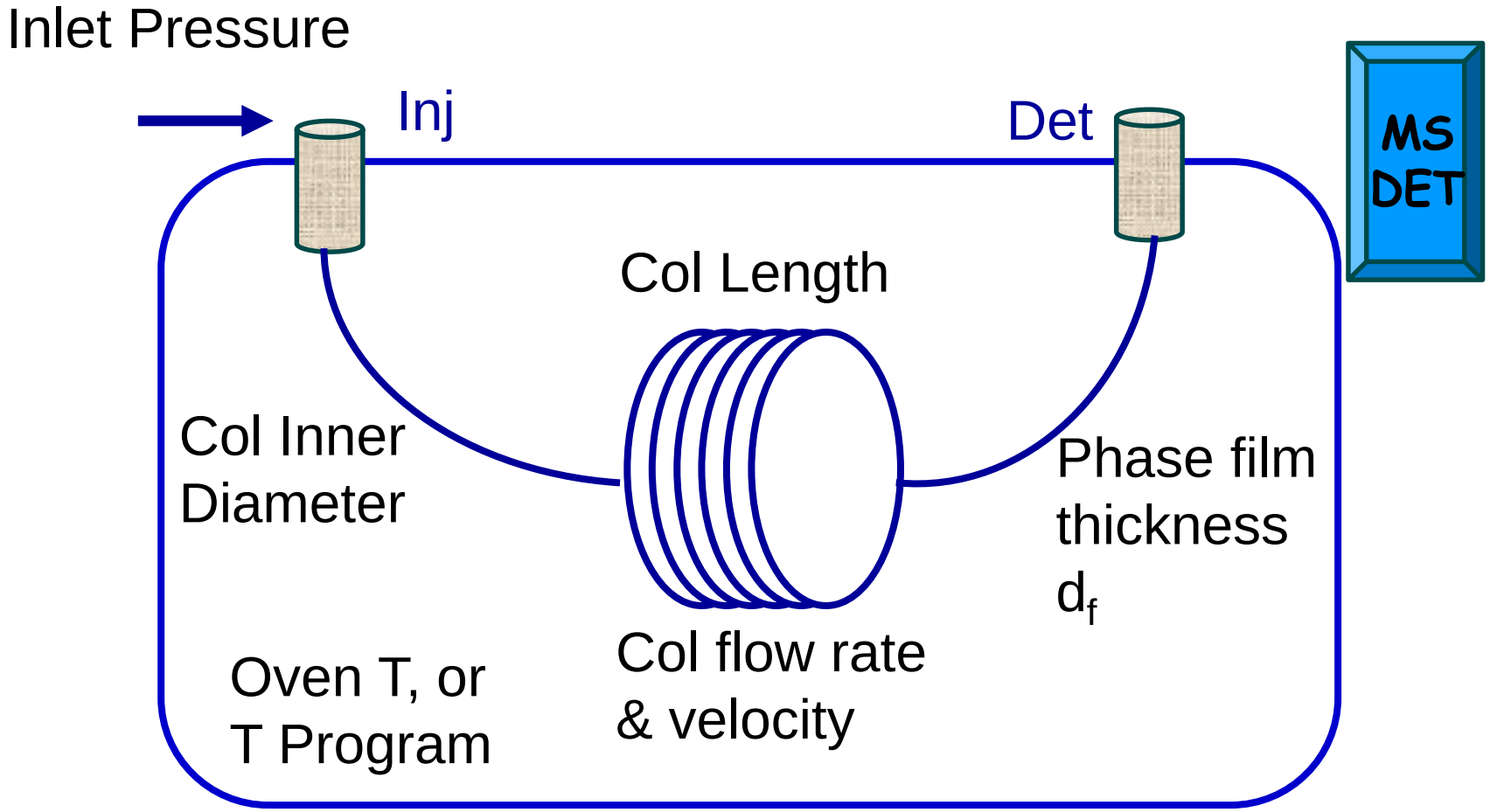
   (a) flow rate optimisation ***(choose best ū).***

   (b) sample size decreased (**does it improve R_S?)**

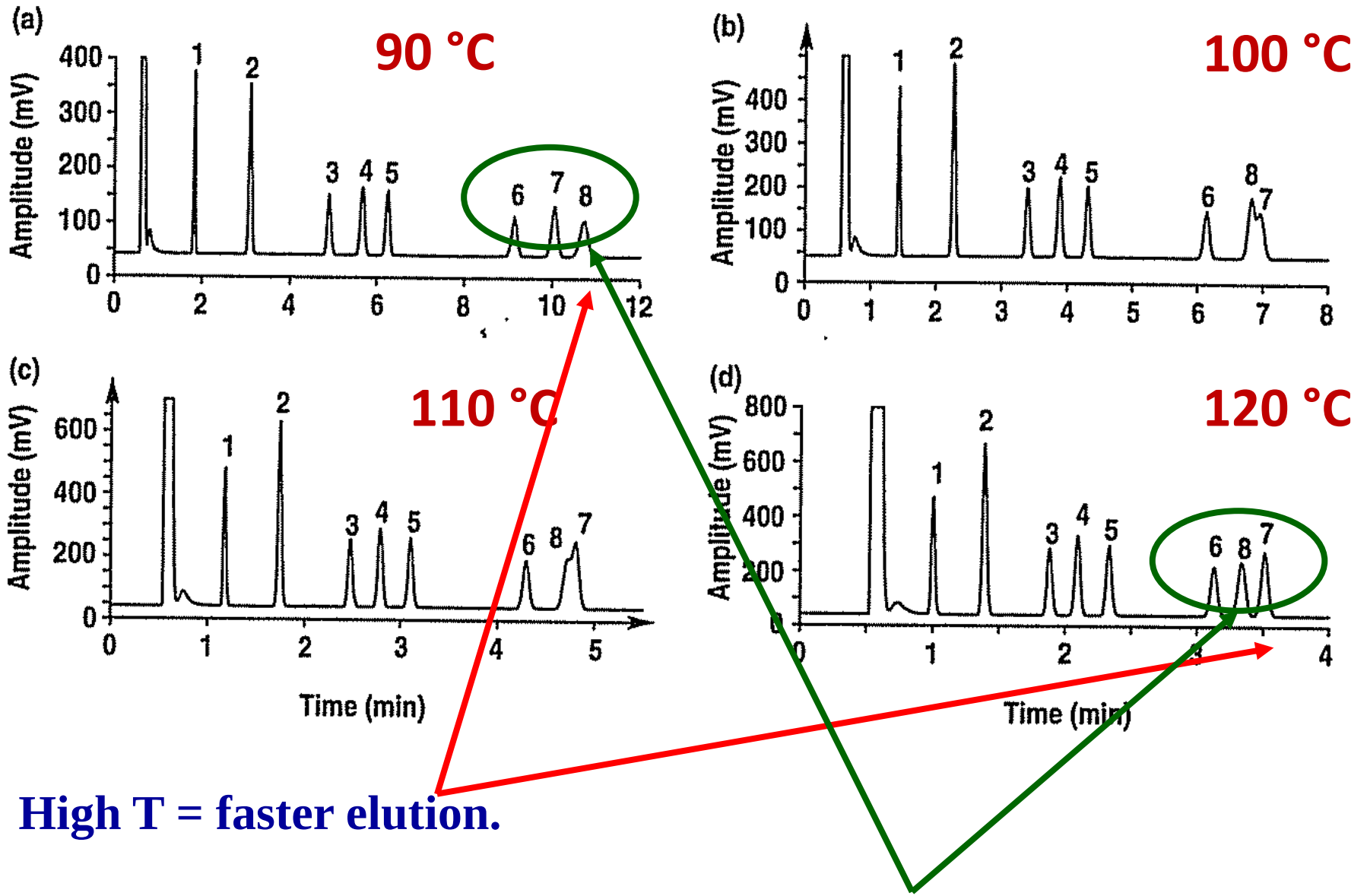
   (c) different mobile phase (LC) ***(change k)***

   (d) col T increased (LC) ***(C_M term)***

   (e) col T decreased (GC) ***(decrease B/u term)*****. Does not always work...**
   Depends on T-dependence of k. BUT is good for chiral

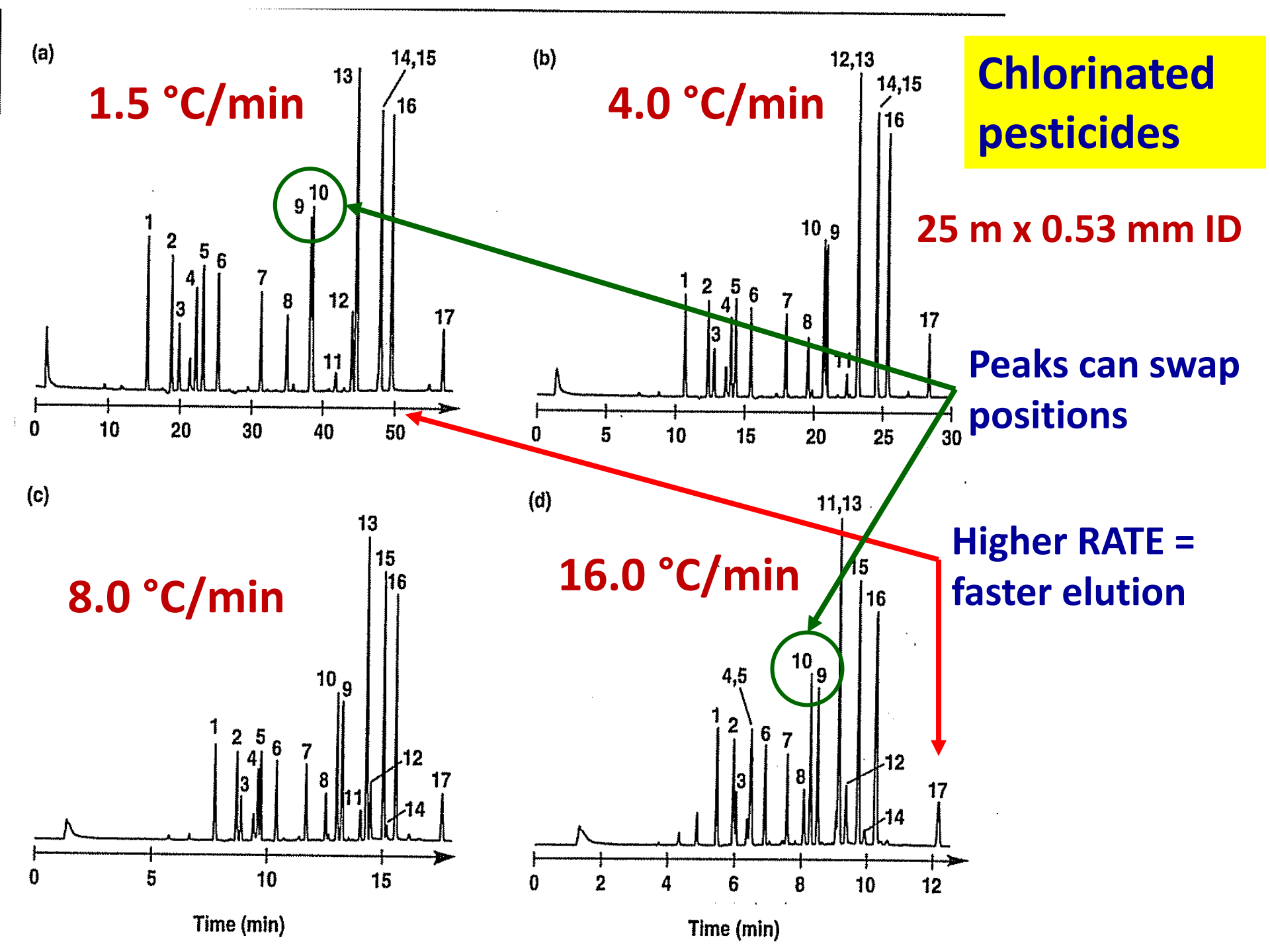


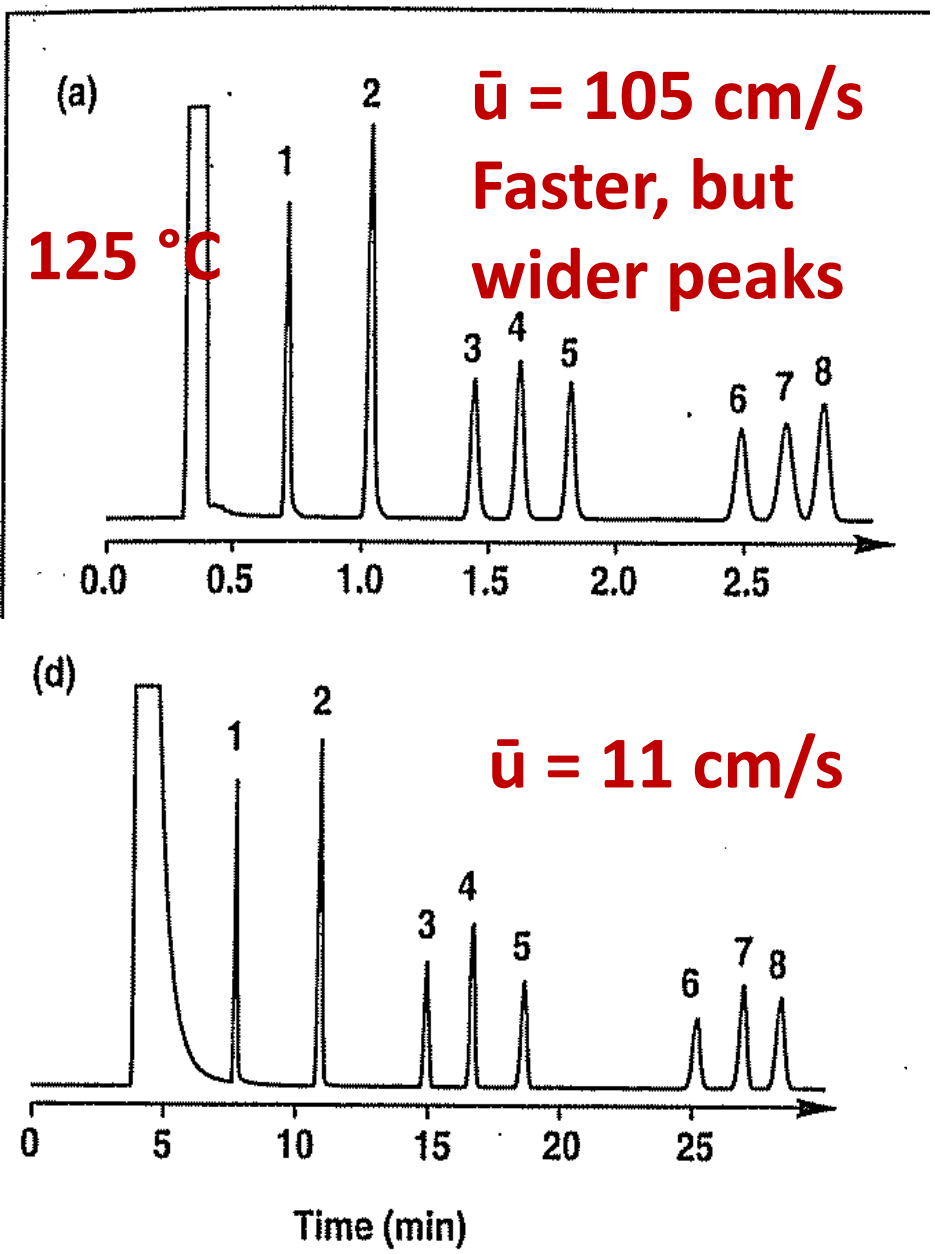
See some data from “GC in the Fast Lane”: John Hinshaw, LC-GC Magazine, vol 13, (1995) 994.



High T = faster elution.

Compounds CAN swap positions / alter relative retention.


Chlorinated pesticides
25 m x 0.53 mm ID
Peaks can swap positions
Higher RATE = faster elution
(a)
1.5 °C/min
(b)
4.0 °C/min
(c)
8.0 °C/min
(d)
16.0 °C/min
Time (min)
Time (min)



125 °C

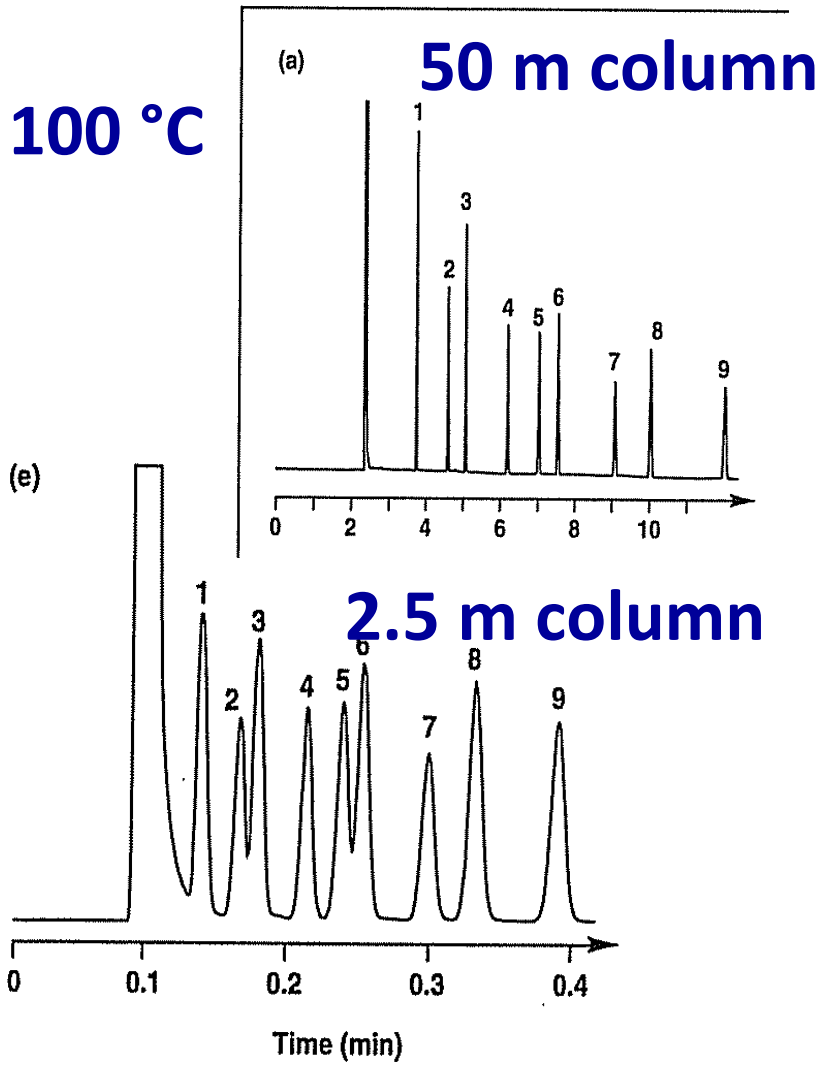
ū = 105 cm/s

Faster, but wider peaks

ū = 11 cm/s

Efficiency (h) varies with flow

The k value stays the same



100 °C

50 m column

2.5 m column

Longer col = more efficient

The k value stays the same

Method Translation – A Route to Faster Analysis with Same Rs

The current trend is towards fast GC
faster operation is beneficial if done correctly.
Agilent Technologies and RESTEK - free programs called
Method Translation
Convert a **SLOWER** method to a **FASTER** method
Gives essentially the same quality separation – Shorter time.
Usually- **shorter**, narrower bore column, **higher** flow velocity, **faster** temp program
Investigate this software to show how different settings can save time whilst giving the same resolution. E.g. shorter and narrower bore columns. And we can compare N_2, H_2 and He carrier.

https://www.restek.com/ezgc-mtfc

EZGC® Method Translator

Carrier Gas	Original	Translation	
	Helium	Helium	
Column			
Length	30.00	15.00	m
Inner Diameter	0.25	0.25	mm
Film Thickness	0.25	0.25	µm
Phase Ratio	250	250	
Control Parameters			
Column Flow	1.40	1.40	mL/min
Average Velocity	42.74	60.44	cm/sec
Holdup Time	1.17	0.41	min
Inlet Pressure psi	11.42	3.77	psi
Outlet Pressure (abs)	0.00	0.00	psi

Oven Program

○ Isothermal
◉ Ramps

Number of Ramps (1-4): 1

	Ramp Rate (°C/min)	Temp (°C)	Hold Time (min)	Ramp Rate (°C/min)	Temp (°C)	Hold Time (min)
		40	1		40	0.35
	8.5	330	1	24	330	0.35

Run Time	36.12	12.78	min
Speed		2.83	x

Translate an original GC separation to a faster analysis – SAME resolution

** Use Method Translation to investigate narrower bore columns also

2.8 x faster

EZGC® Flow Calculator

Carrier Gas

		Helium	
Column			
Length		30.00	m
Inner Diameter		0.25	mm
Film Thickness		0.25	µm
Temperature		40.00	°C
Control Parameters			
Column Flow	Optimum Range 1.4 to 2.0 mL/min	1.40	mL/min
Average Velocity		42.74	cm/sec
Holdup Time		1.17	min
Inlet Pressure	psi	11.42	psi
Outlet Pressure (abs)		0.00	psi
Inlet			
Temperature		250.00	°C
Liner Volume		1.00	mL
Flow		1.40	mL/min
Splitless Valve Time		1.1 to 1.5	min

RESTEK offers a FLOW CALCULATOR i.e. flow velocity & volume for a given pressure and column geometry

* what happens if you Change GC Temp? Change col Length?

GC T. Same flow vol = must ↑ pressure
Same P but ↑ T = flow velocity ↓
Col L ↑ @ same P = large ↑ in t_M.

A favourite Equation!

Using the Equation

$$K = k\beta$$

$$K = \frac{C_S}{C_M} = k\beta = \frac{t'_R}{t_M}\,\frac{r}{2d_f}$$

See the uploaded file