Chromatographic Methods

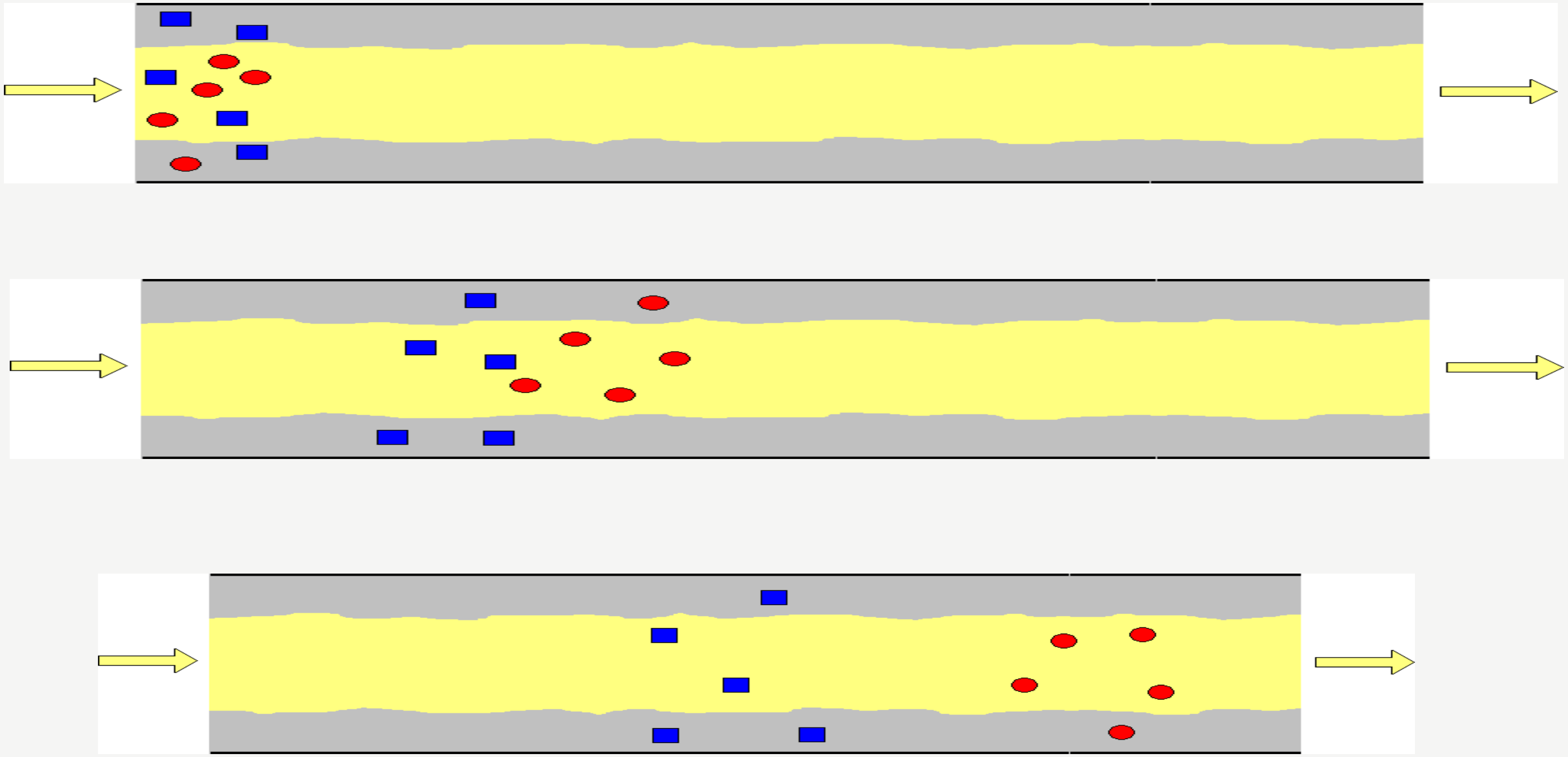
General Fundamentals of Chromatographic Methods

Liquid Chromatography (LC)

Gas Chromatography (GC)

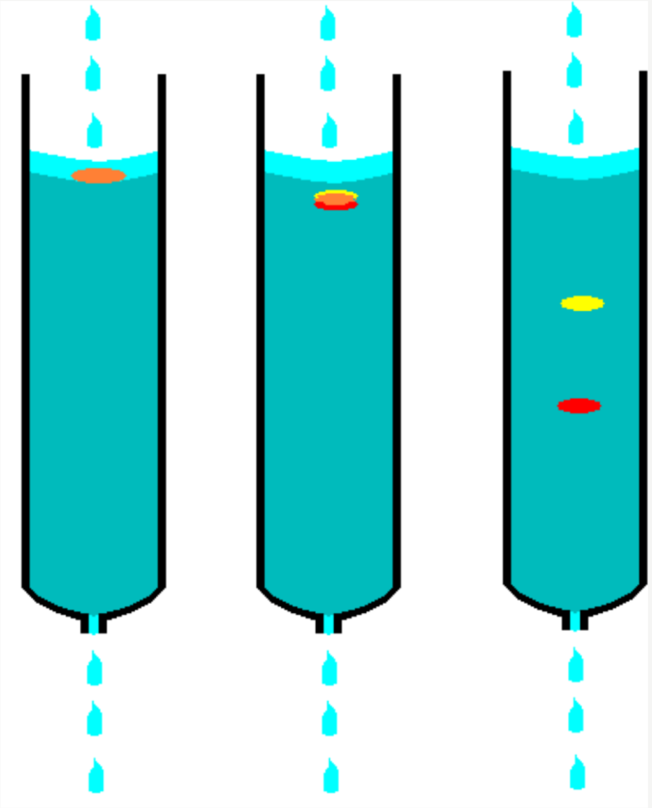
Chromatographic Methods

Separation of a mixture by differential interaction of its components between a STATIONARY PHASE (liquid or solid) and a MOBILE PHASE (liquid or gas).



Chromatographic Methods

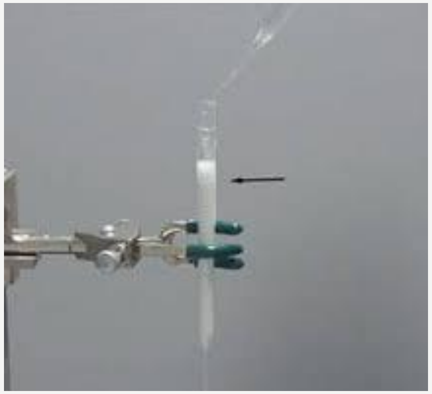
Column chromatography (most common): the stationary phase is contained in a column through which it is forced to pass the mobile phase)



Column Chromatography

Two types of column:

1) **Packed columns**

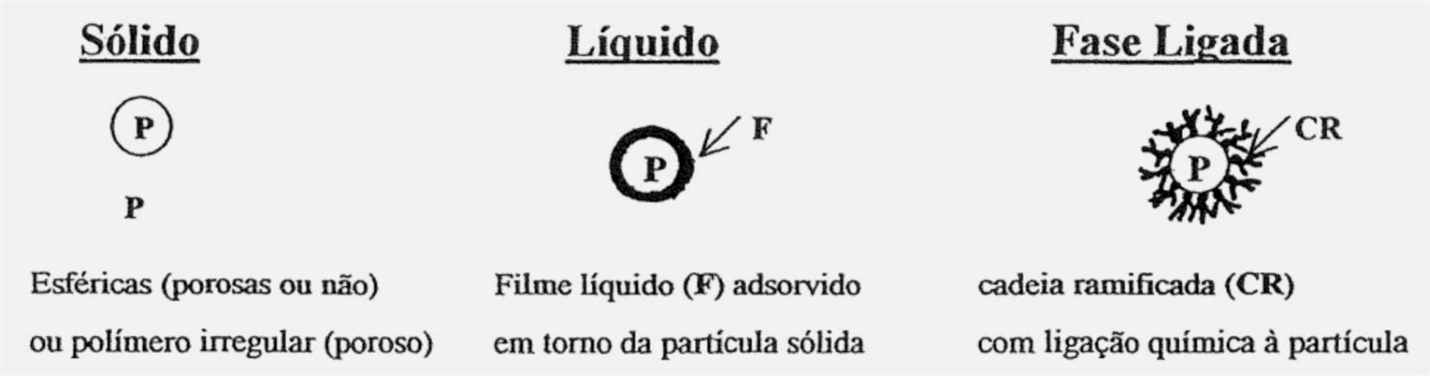


2) **Capillary columns**

Packed columns

Tube made of a chemically inert material (glass, steel…), with a total volume $Vt = \pi r^2 L$ (L is the length of the column and r its internal radius), filled with finely divided solid particles, occupying fixed positions in the column with a volume Vs. The mobile phase occupies the interstitial spaces between particles, which defines Vm.

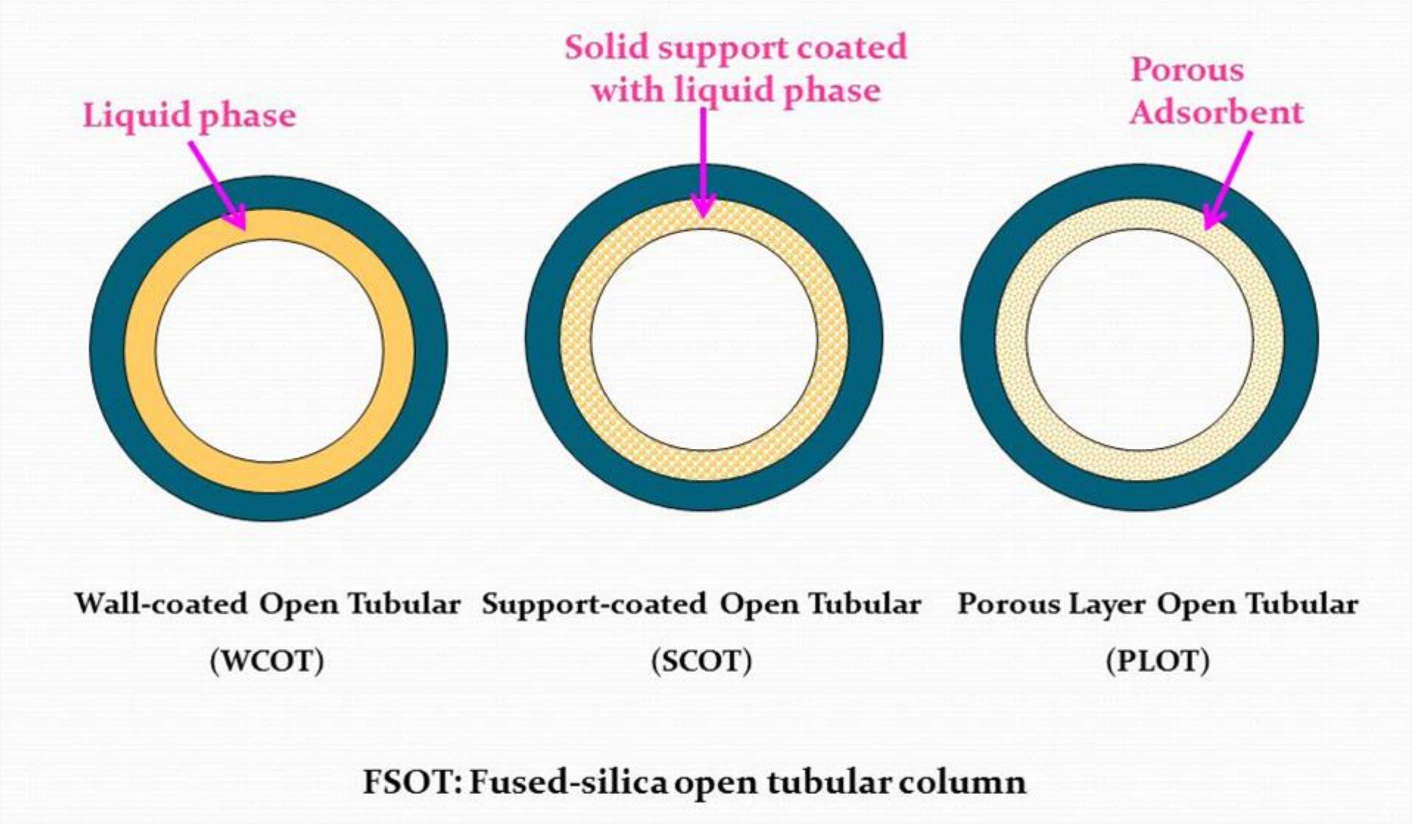
Packing Variety



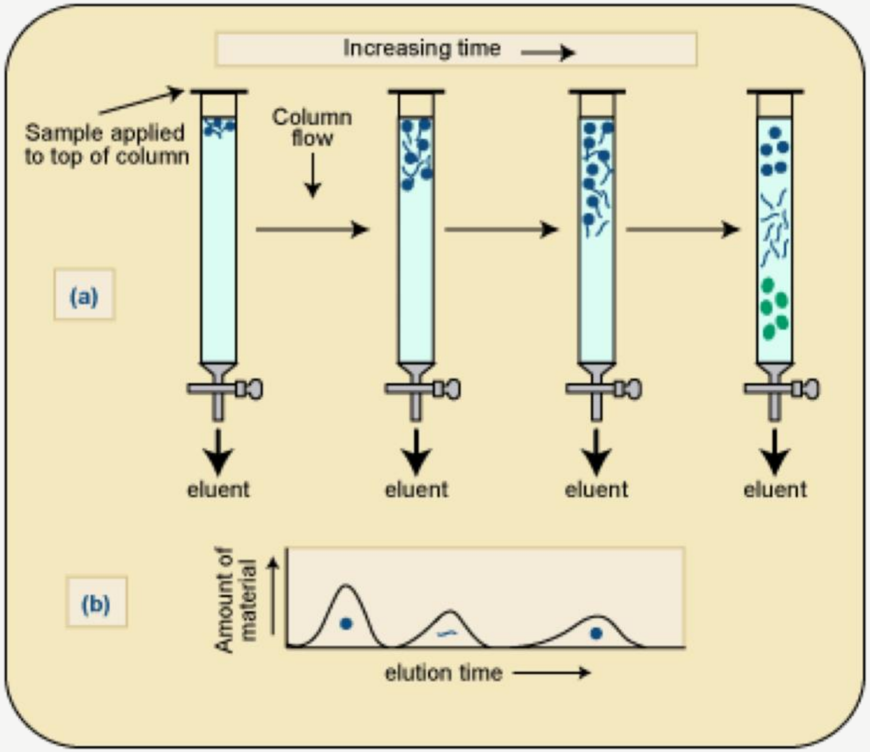
Column Chromatography

Types of Capillary Columns

Capillary tube coated internally and evenly by the stationary phase.

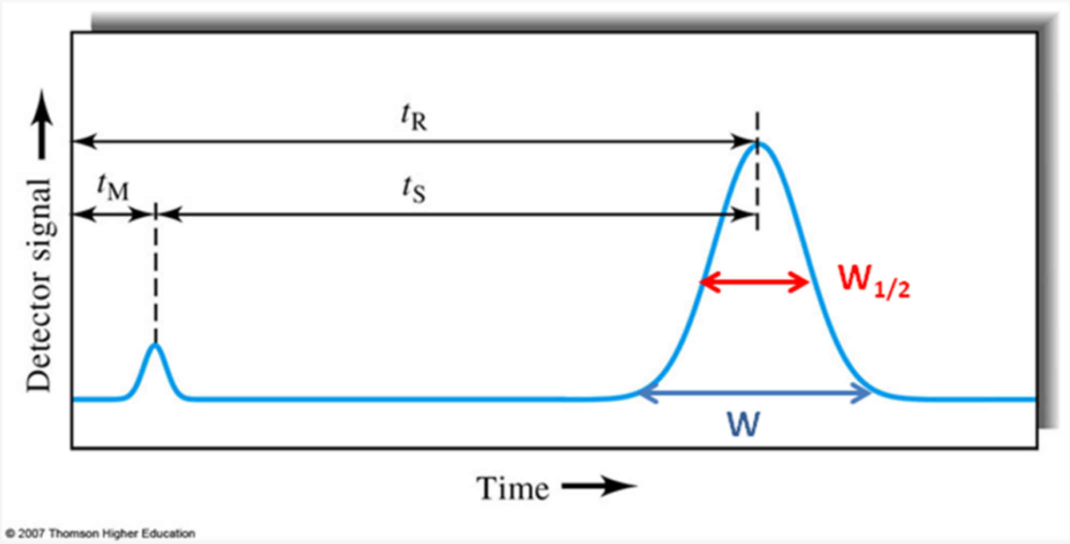


Elution on column chromatography



Column Chromatography

Chromatogram

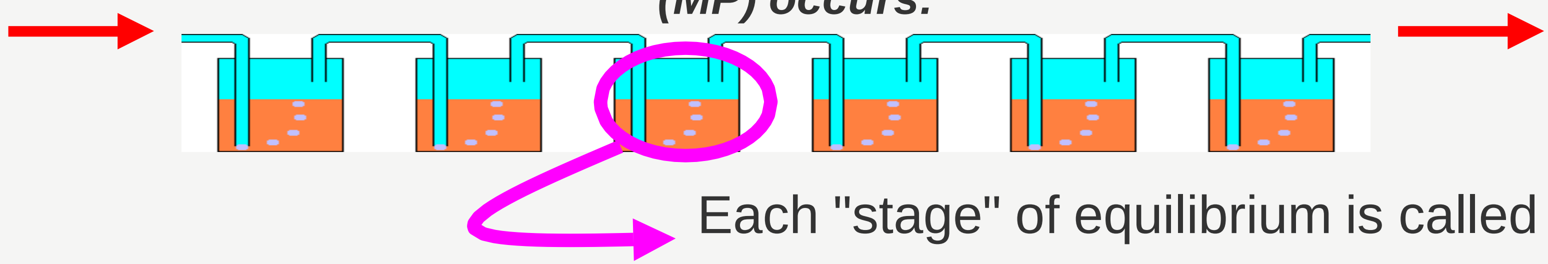


QUALITATIVE ANALYSIS: Retention Time tr (s) or Retention Volume Vr (mL) (Vr = F tr) where F is the mobile phase volumetric flow rate.

QUANTITATIVE ANALYSIS: Peak Area.

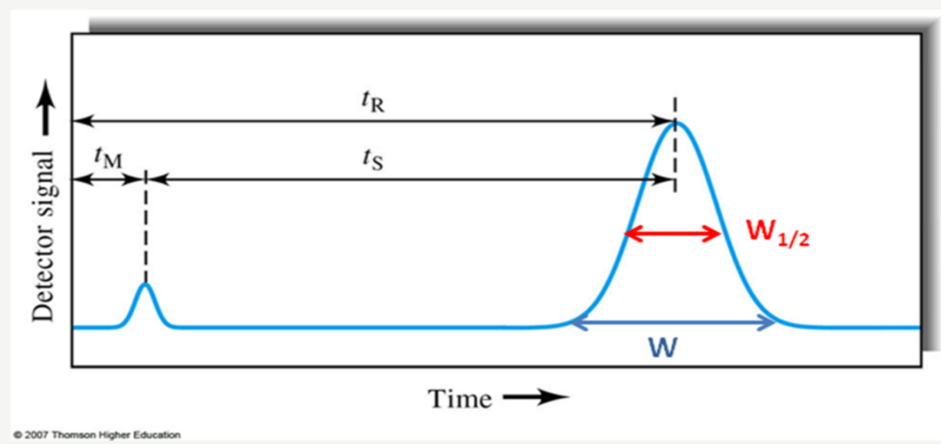
Column Chromatography

Assuming the chromatographic column as a series of independent stages where the balance between the analyte, the stationary phase (SP) and the mobile phase (MP) occurs:



Each "stage" of equilibrium is called a **THEORETICAL PLATE**

- **t_r** - retention time (s)
- **$W_{1/2}$** - width at half height (units of tr)
- **W** - triangle base width (tr units)
- **t_M** - retention time (s) of an unretained substance, i.e., elution time of the eluent.



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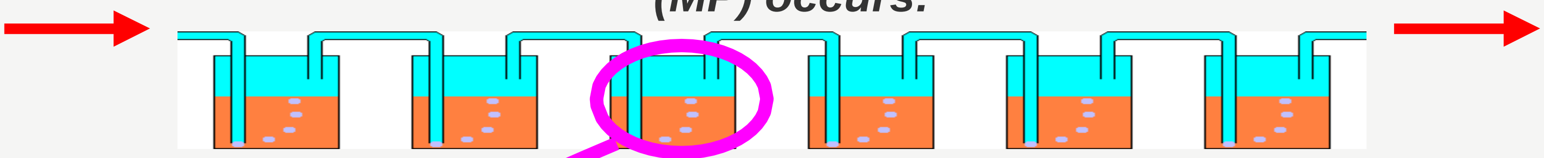


Each "stage" of equilibrium is called a **THEORETICAL PLATE**

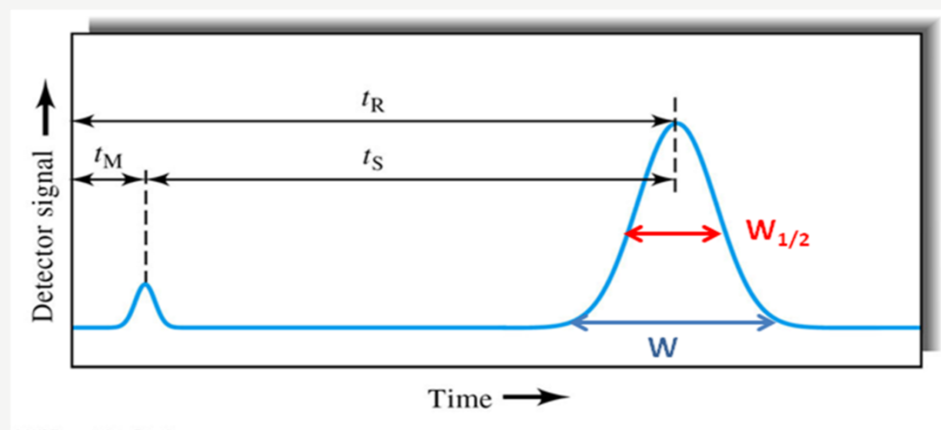
Column of length L (cm) formed by a number N of theoretical plates of height H (height equivalent to a theoretical plate):

$$N = L/H$$

Calculation of N from the chromatogram (assuming the peaks are Gaussian)

$$N = 16\,(t_R/W)^2$$

$$N = 5.54\,(t_R/W_{1/2})^2$$



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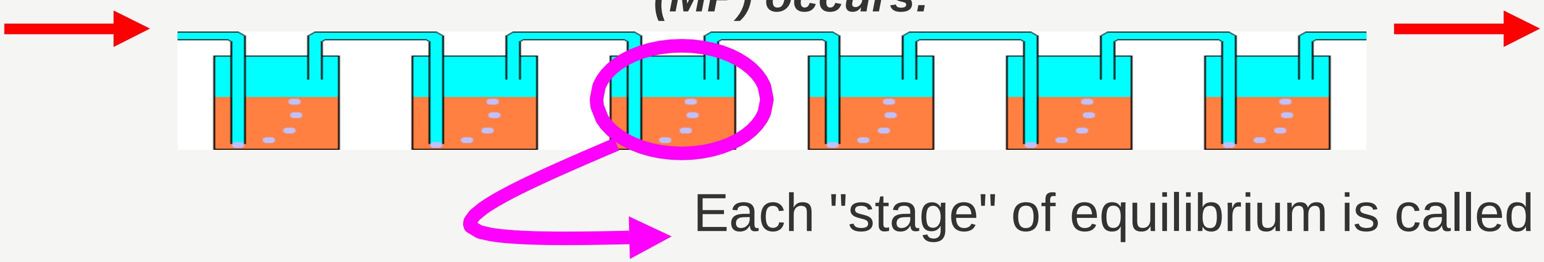

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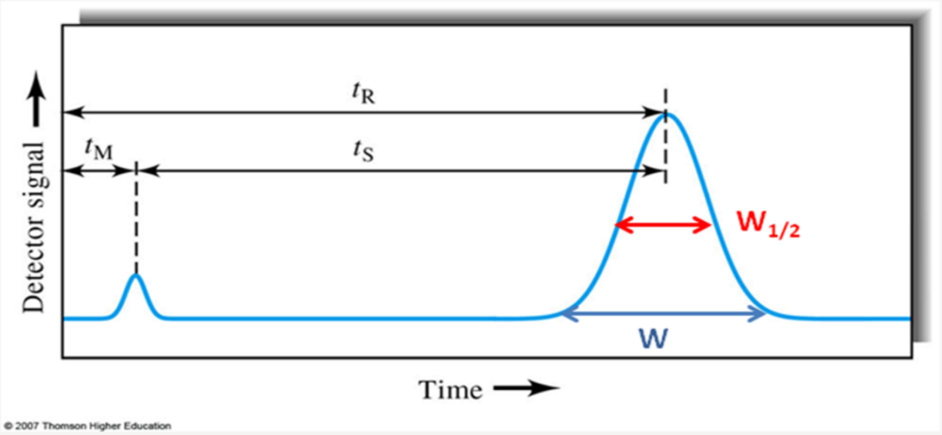
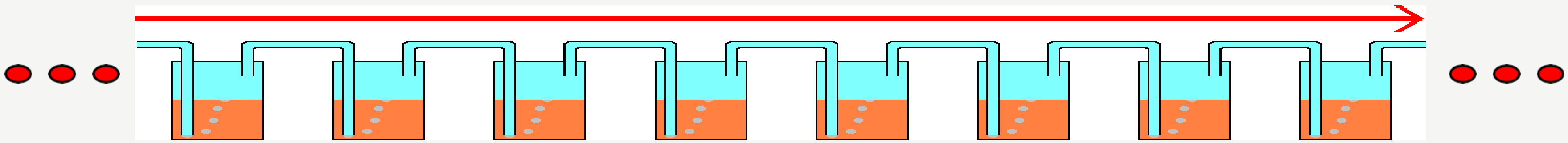
$$N = 16\,(t_R/W)^2$$

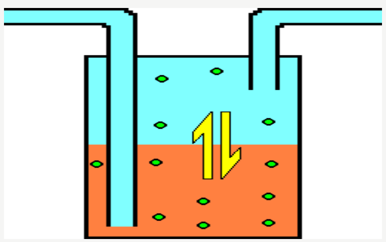


↑ t_R, ↓ w → ↑ N → Column more efficient

Column Chromatography

An analyte distribution equilibrium between SP and MP occurs.





$$K_C = \frac{[A]_S}{[A]_M}$$

K_C = Distribution Constant

$[A]_S$ = analyte concentration in SP

$[A]_M$ = analyte concentration in the MP

↓ ***Affinity for the SP*** → ↓ **$[A]_S$**

LESS RETENTION!!!

Column Chromatography

Parameters that characterize a chromatographic elution

$$A\,(m) \leftrightharpoons A\,(s)$$

- **Distribution constant:**

$Kc = [A]_s / [A]_m$ $=(n_s/V_s)/(n_m/V_m)$

V_m: Volume of the mobile phase ($V_m = t_m F$)

n_s e n_m: moles of A in each of the phases

- **Selectivity factor:**

$\alpha = k_2/k_1 = (t_2-t_m)/(t_1-t_m)$

for two solutes 1 and 2 where

2 is the most retained

(ideally $\alpha > 1$)

- **Retention factor:**

Column capacity to retain a component of the sample, i.e., is the measure of retention in the stationary phase.

The more a component is retained by the column, the greater is its k.

$k = n_s/n_m = Kc\, V_s / V_m$ (Ideally $1<k<10$)

Relationship between retention factor and

retention time

k = stationary phase time/mobile phase time

$k = (t_r-t_m)/t_m = (V_r-V_m)/V_m$

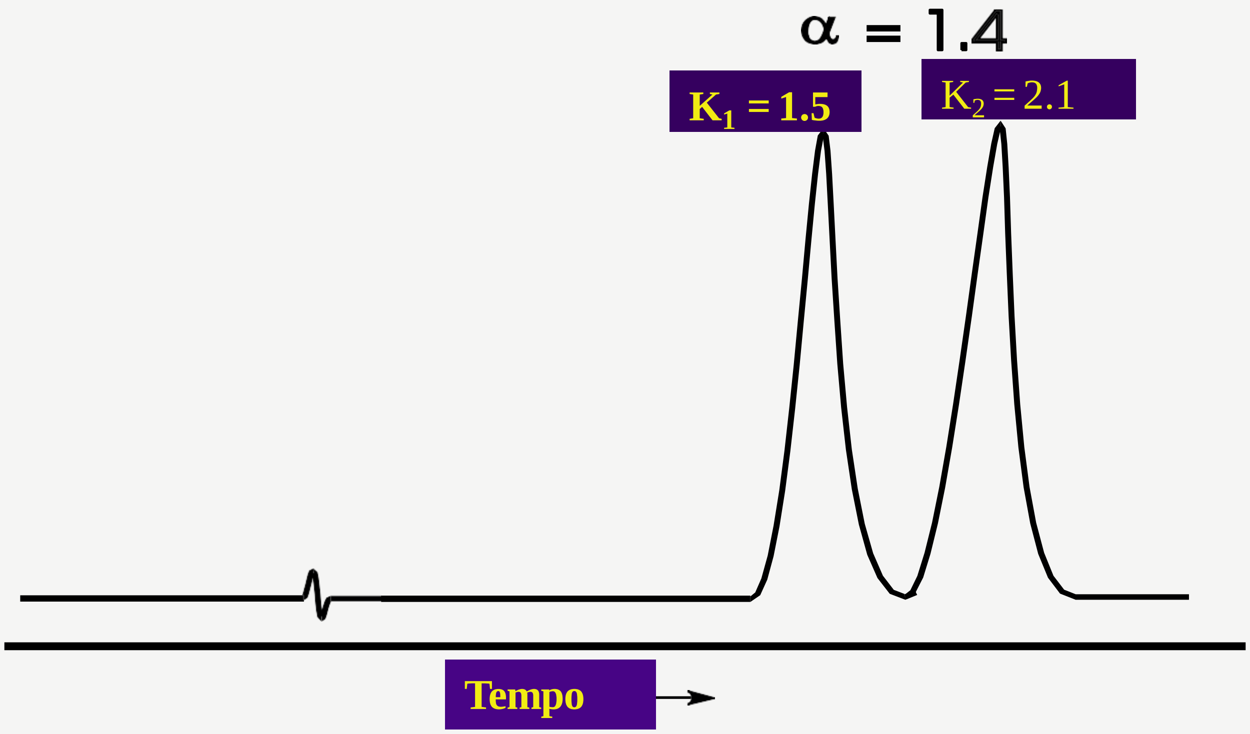
Vr – Retention volume for the solute

Selectivity (α)

$\alpha = k_2/k_1$

α = relative position of 2 peaks

Ideally: $\alpha > 1$



Resolution (Rs)

Rs = actual peak separation

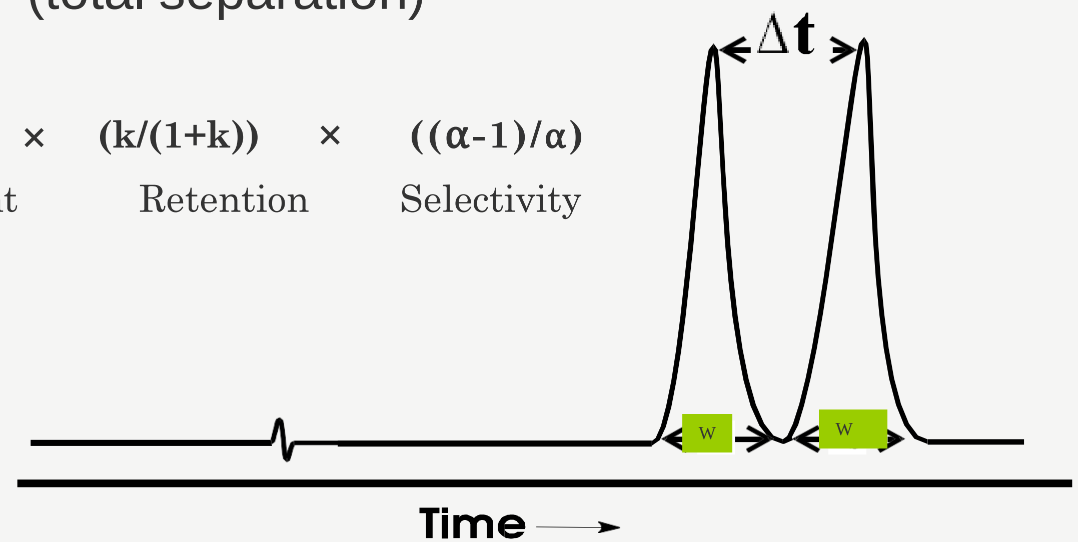
$Rs = 2\,(t_{R2} - t_{R1}) / (w_1 + w_2)$

$Rs = \Delta t_R / W_{average} = 0.589\,\Delta t_R / W_{1/2\ average}$

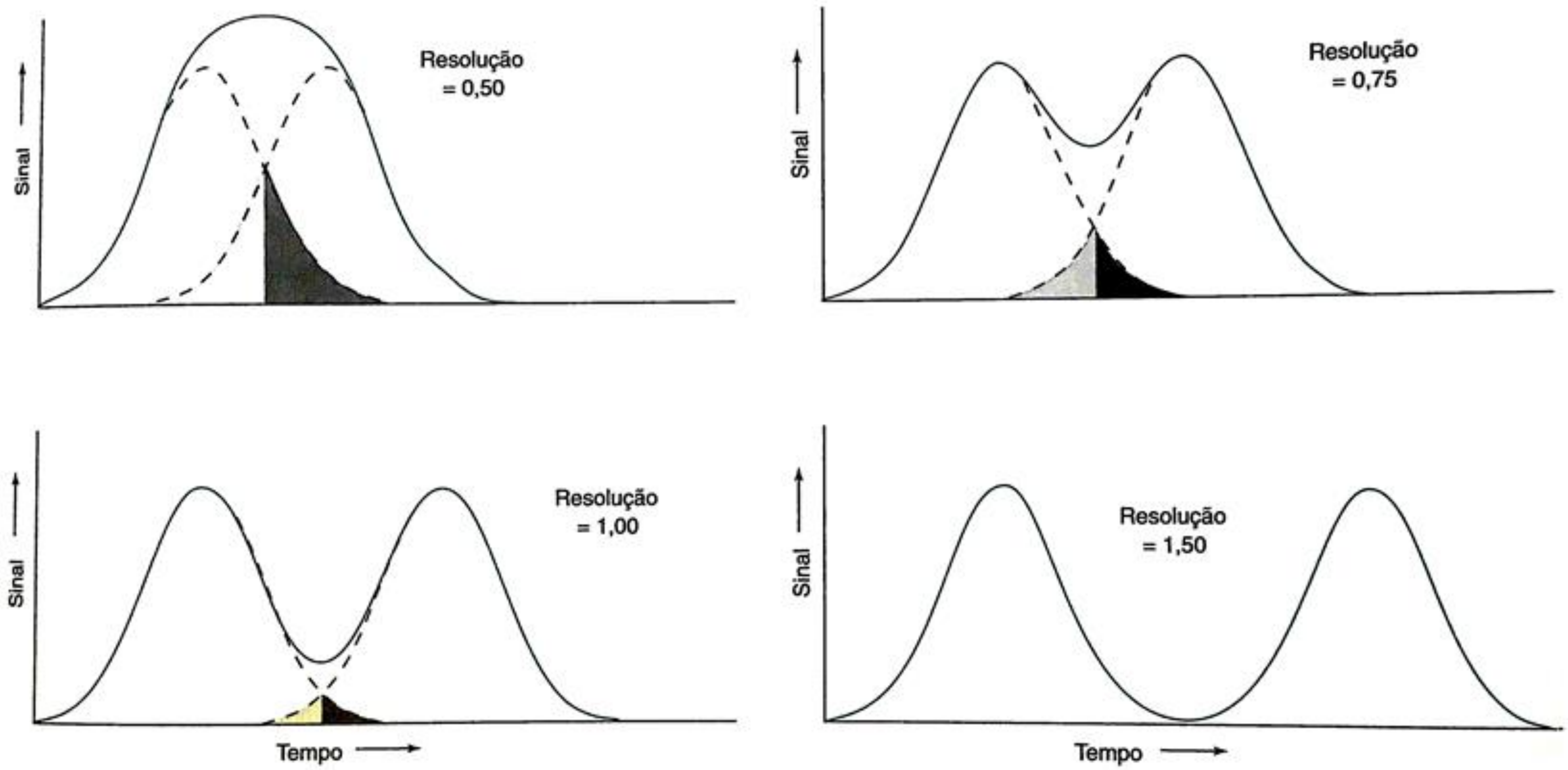
Rs = 0 (no separation), Rs = 1 (partial separation), Rs = 1.5 (total separation)

$$R_s = ((\sqrt{N})/4) \times (k/(1+k)) \times ((\alpha - 1)/\alpha)$$

Efficient   Retention   Selectivity

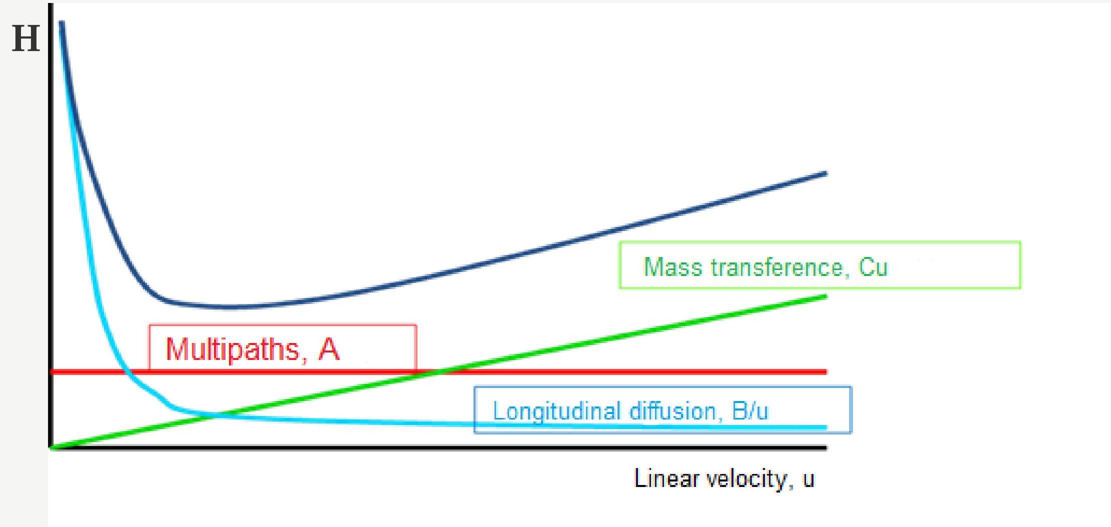


Resolution (Rs)



Optimizing the efficiency of a column

Kinetic Theory - The equivalent height of a theoretical plate H is a function of the average linear velocity u.



Algebraic relations between H and u:

- Packed Columns: van Deemter equation

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

(A, B, C = constants)

- Capillary Columns: Golay equation

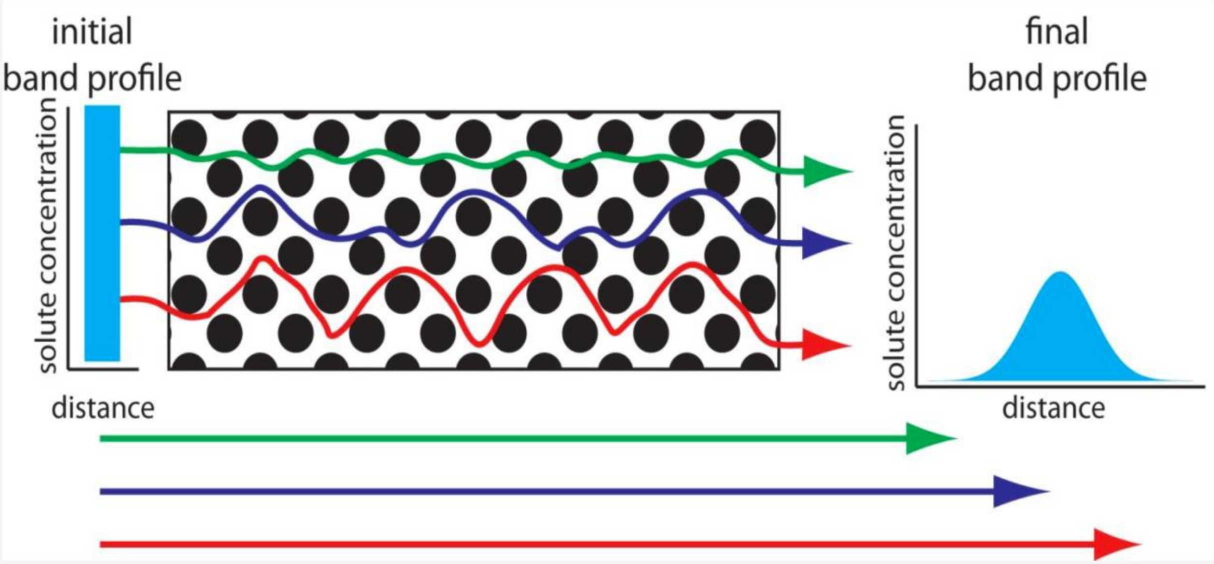
$$H = \frac{B}{\bar{u}} + (C_M + C_S) \cdot \bar{u}$$

(B, C_M, C_S = constants)

Optimizing the efficiency of a column

Kinetic Theory - Multipath Terms(A)

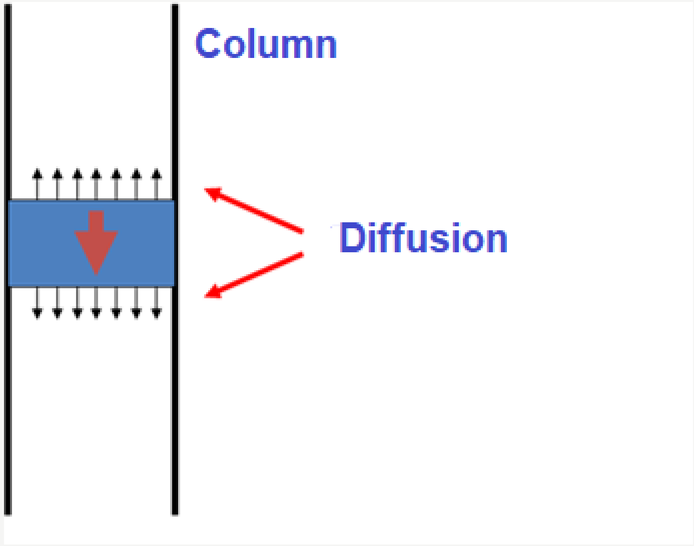
Multipath term A (independent of u): W increases with tR and/or L due to the different paths the species can take inside the column.

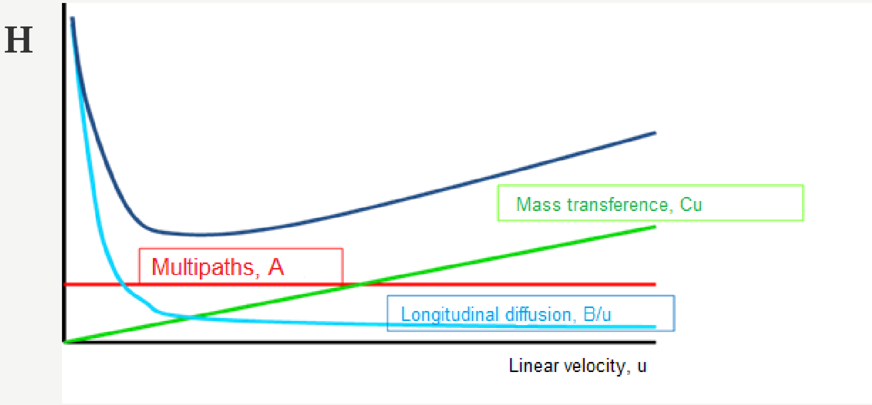


Optimizing the efficiency of a column

Kinetic Theory - Longitudinal Diffusion Terms (B/u)

Longitudinal Diffusion Term (B/u) refers to the diffusion of a solute into the mobile phase. B = f (D) due to the passage of each of the species in the mixture from the more concentrated zone to the more diluted ones, and therefore proportional to the diffusion coefficient D.





Optimizing the efficiency of a column

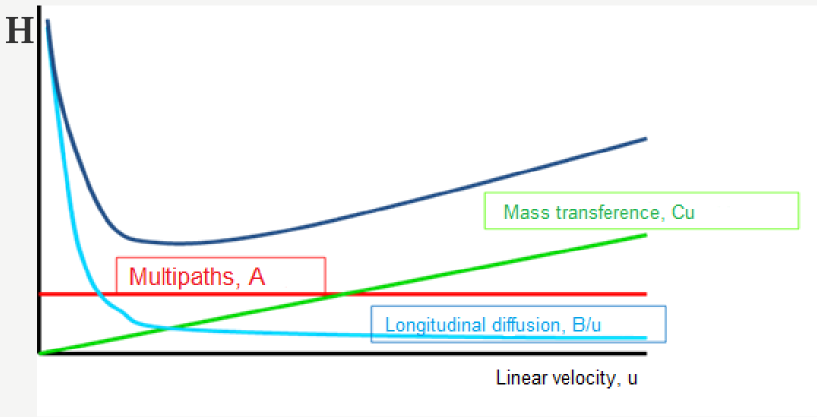
Kinetic Theory - Mass Transfer Term (C.u)

($C.u = C_S\, u + C_M\, u$)

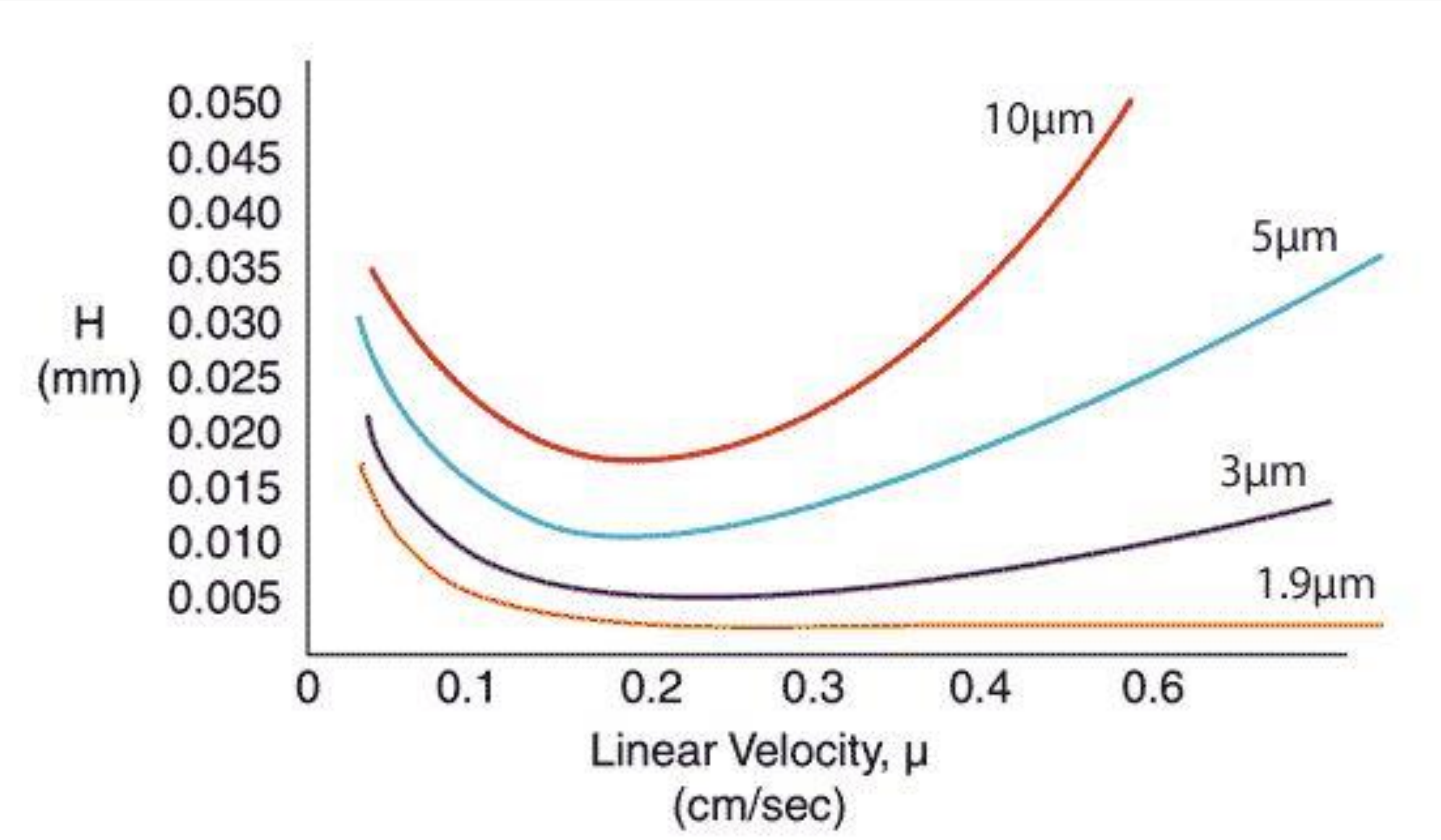
The higher u, the shorter the time for mass transfer to take place, the lower the efficiency.

In the case of liquid chromatography, the term of mass transfer is the most important, whereby H is minimized by decreasing particle size, using low flow rates.

In the case of gas chromatography, the term of longitudinal diffusion is the most important, so H is minimized by using high flow rates.



Optimizing the efficiency of a column



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Good separation in the shortest possible time

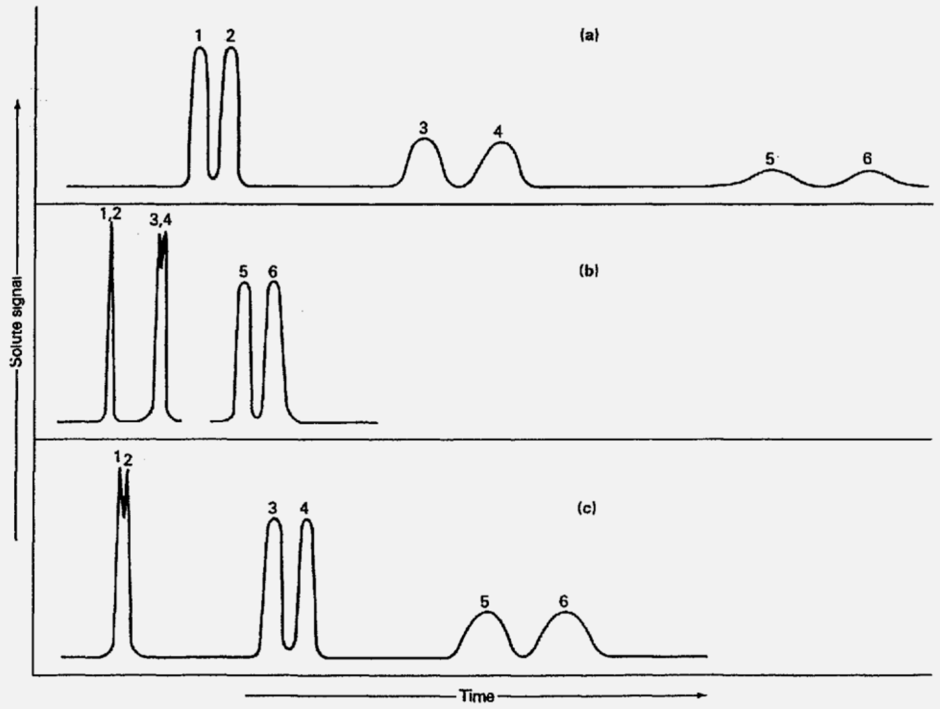

Illustration of the general elution problem in chromatography.

WHAT CAN VARY:

-Column length (varies N)

-Mobile phase flow (varies H)

-Packing particle size (varies H)

-Mobile phase composition (varies k)

Chromatographic Methods - Quantitative Analysis

The determination of the concentration of the species is based on the peak area measurements, A, proportional to the number of injected moles

Area (A) = constant × number of moles injected = constant x $\mathbf{V_{inj}}$ x **C = constant’** x **C**

where the constant is a parameter that depends on experimental conditions, such as speed u and type of detector used, C is the concentration of the species with peak area A, and Vinj is the injected volume (in the order of µL).

Exercise 1

A mixture of benzene, toluene and methane was injected into a gas chromatograph (GC). Methane produced a peak after 42s, while benzene required 251s and toluene eluted in 333s. Determine:

- the retention factor (k) for each solute.
- the selectivity factor (α).

$$k = \frac{t_r - t_m}{t_m}$$

$$\alpha = \frac{k2}{k1}$$

Exercise 1

PROBLEM SOLVING

- The retention factors are:

$$Benzeno: k = \frac{t_r - t_m}{t_m} = \frac{251 - 42}{42} = 5{,}0$$

$$Tolueno: k = \frac{t_r - t_m}{t_m} = \frac{333 - 42}{42} = 6{,}9$$

- The selectivity factor (α) is:

$$\alpha = \frac{\mathrm{k}(tolueno)}{\mathrm{k}(benzeno)} = \frac{333 - 42}{251 - 42} = 1{,}39$$

TÉCNICO LISBOA

Exercise 2

A peak with a retention time of 407s has a width at half height of 7.6s. A neighboring peak is eluted 17s later with $w_{1/2}$ = 9.4 s.

(a) Determine the resolution for these two components.

(b) What retention time difference is required for an adequate resolution of 1.5 s?

TÉCNICO LISBOA

Exercise 2

PROBLEM SOLVING

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(a)

$$\text{Resolution} = \frac{0{,}589\Delta t_r}{w_{1/2_{méd}}} = \frac{0{,}589 \times 17\ s}{\frac{7{,}6\ s + 9{,}4\ s}{2}} = 1{,}18$$

(b)

$$1{,}5 = \frac{0{,}589\Delta t_r}{\frac{7{,}6\ s + 9{,}4\ s}{2}} \rightarrow \Delta t_r = 21{,}6$$