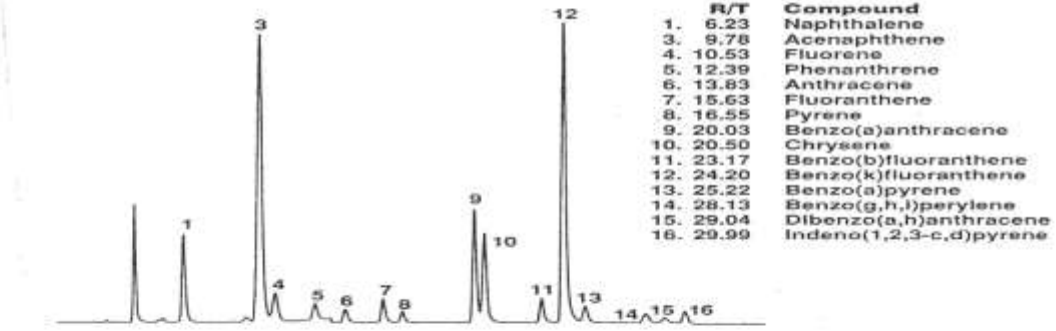
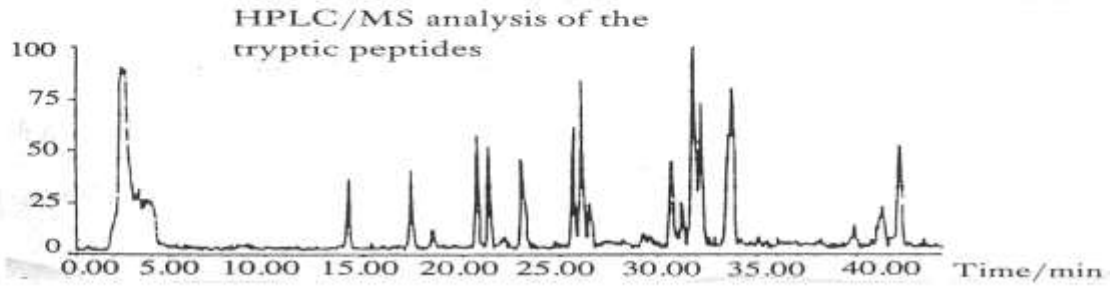
Why are Analytical Chemists so interested in Separations?

1. Interference removal
2. Contamination removal
3. Separation of complex mixtures into their individual components
4. Concentrate analyte

Fluorescence Chromatogram of Water Spiked Sample



	R/T	Compound
1.	6.23	Naphthalene
3.	9.78	Acenaphthene
4.	10.53	Fluorene
5.	12.39	Phenanthrene
6.	13.83	Anthracene
7.	15.63	Fluoranthene
8.	16.55	Pyrene
9.	20.03	Benzo(a)anthracene
10.	20.50	Chrysene
11.	23.17	Benzo(b)fluoranthene
12.	24.20	Benzo(k)fluoranthene
13.	25.22	Benzo(a)pyrene
14.	28.13	Benzo(g,h,i)perylene
15.	29.04	Dibenzo(a,h)anthracene
16.	29.99	Indeno(1,2,3-c,d)pyrene



Chromatography

chromatography: chroma (colour) + graphy (write)
"colour-writing"

IUPAC defines chromatography as:
A method used primarily for the separation of components of a sample, in which the components are distributed between two phases, one of which is stationary while the other moves. The stationary phase may be a solid, or a liquid supported on a solid, or a gel. The stationary phase may be packed in a column, spread as a layer, or distributed as a film, etc. In these definitions, "chromatographic bed" is used as a general term to denote any of the different forms in which the stationary phase may be used. The mobile phase may be gaseous or liquid.

Tswett's contribution to chromatography

Introduced a matchless procedure for the resolution of mixtures of substances with similar chemical properties and revealed the basic principles of the method.

If a petroleum ether solution of chlorophyll is filtered through a column of an adsorbent (I use mainly calcium carbonate which is stamped firmly into a narrow glass tube), then the pigments, according to the adsorption sequence, are resolved from top to bottom into various colored zones. . . . Like light rays in the spectrum, so the different components of a pigment mixture are resolved on the calcium carbonate column according to a law and can be estimated on it qualitatively and quantitatively. Such a preparation I term a *chromatogram*, and the corresponding method, the *chromatographic method*.

It is self-evident that the adsorption phenomena described are not restricted to the chlorophyll pigments, and one must assume that all kinds of colored and colorless chemical compounds are subject to the same laws.

[Ref: Tswett, Michael, 1906, as translated and quoted in *J. Chem. Ed.* **1959**, *36*, 144 and *Ibid* **1967**, *44*, 235.]

First published article on chromatography 1906 (in German)
J. of Chemical Education, 44, 238, (1967).

Recommendations on Nomenclature for Chromatography

(Given by International Union of Pure and Applied Chemistry; published in *Pure and Applied Chemistry*, vol. 37, p. 447, 1974)

Terms relating to method in General:

Chromatogram

A graphical or other presentation of detector response, effluent concentration, or other quantity used as a measure of effluent concentration, versus effluent volume or time. The term is also applied to the layer or paper after separation has occurred.

Chromatograph *(verb)*

To separate by chromatography.

Chromatograph *(noun)*

The assembly of apparatus for carrying out chromatographic separation.

Eluent

The liquid or gas entering the chromatographic bed and used to effect the separation by elution.

Carrier gas

The term normally used for the eluent in gas chromatography.

Mobile phase

The phase that is moving in the chromatographic bed. It includes the fraction of the sample present in the phase.

Stationary phase

The non-mobile phase in the chromatographic bed, on which the separation depends. For example, in gas-solid chromatography and liquid-solid chromatography the active solid is the stationary phase, and in the gas-liquid and liquid-liquid chromatography the liquid, but not the solid support, is the stationary phase.

Solid support

A solid that holds the stationary liquid phase.

Eluate

The effluent from a chromatographic bed emerging when elution is carried out.

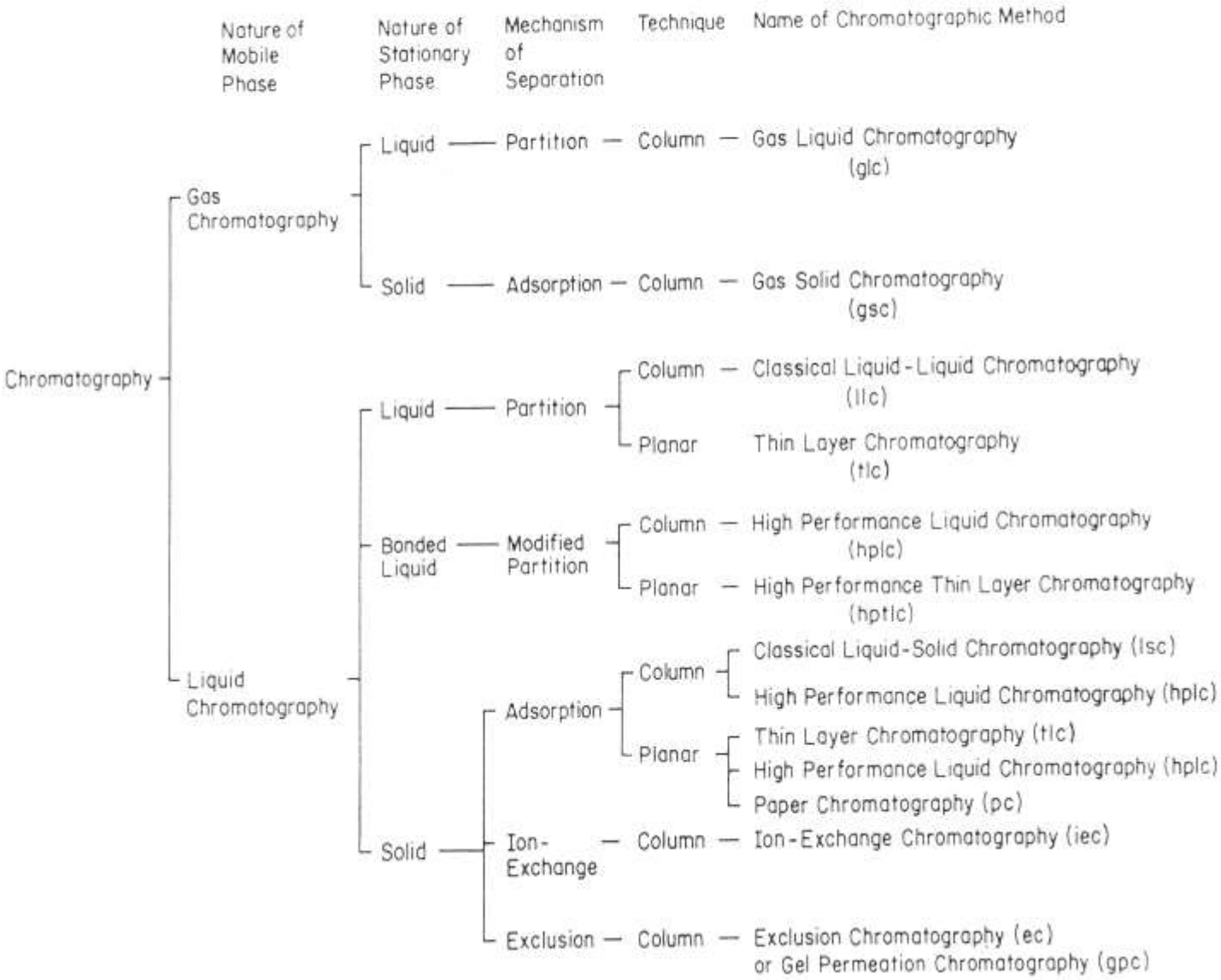
Peak (elution band)

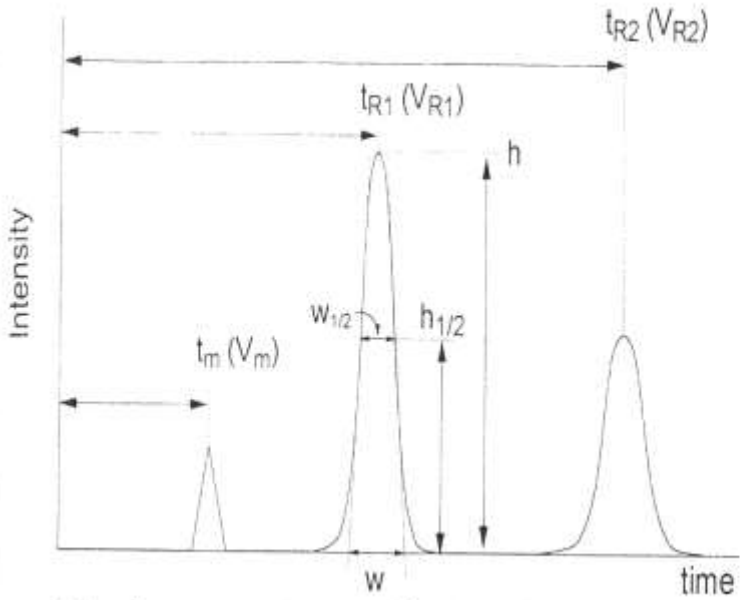
The portion of a differential chromatogram recording the detector response or eluate concentration while a component emerges from the column. If separation is incomplete, two or more components may appear as one unresolved peak.

Baseline

The portion of the chromatogram recorded when only eluent or carrier gas emerges from the column.

Classification of Chromatographic Methods





Retention parameters used for describing bands in column chromatography:

Capacity factor

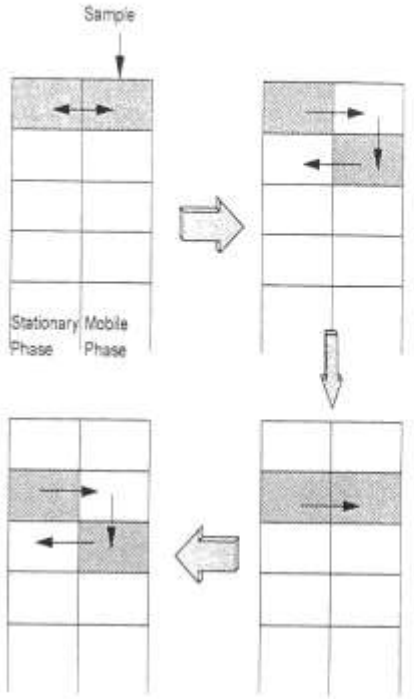
$$k_i' = \frac{t_{Ri} - t_m}{t_m} = \frac{t_{Ri}'}{t_m} \qquad 1 \geq k_i' \geq 10$$

Selectivity factor (seperation factor, relative retention)

$$\alpha_{21} = \frac{k_2'}{k_1'} = \frac{t_{R2} - t_m}{t_{R1} - t_m} = \frac{t_{R2}'}{t_{R1}'} \quad (\alpha \geq 1)$$

Resolution

$$R_S = \frac{t_{R2} - t_{R1}}{0.5(W_1 + W_2)}$$



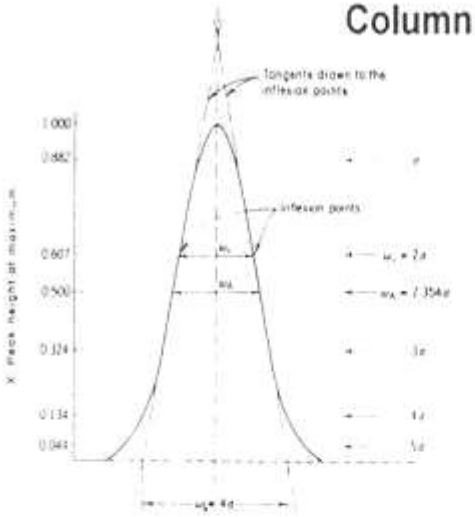
Fundamental Equations in chromatography

$$t_R = t_m\left(1 + K_D \frac{V_S}{Vm}\right)$$

$$k' = K_D\left(\frac{V_S}{V_M}\right) \qquad \alpha_{ij} = \frac{K_{Dj}}{K_{Di}}$$

$$V_R = Vm + K_D V_S$$

Column Efficiency



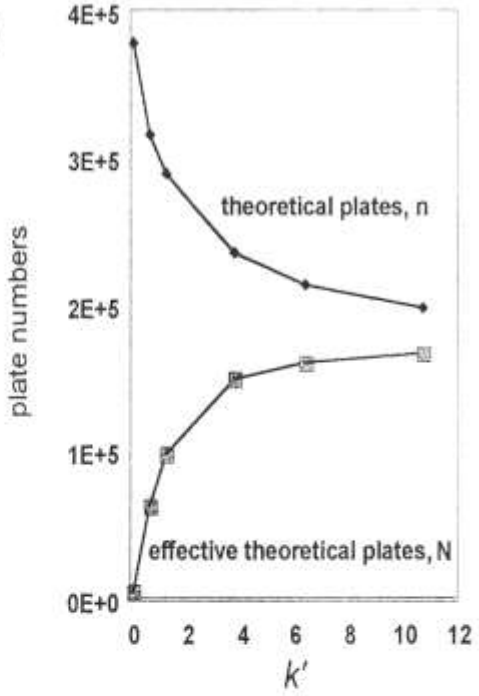
$$n = \left(\frac{t_R}{\sigma}\right)^2 \qquad N = \left(\frac{t'_R}{\sigma}\right)^2$$

$$n = 16\left(\frac{t_R}{W}\right)^2 \qquad N = 16\left(\frac{t'_R}{W}\right)^2$$

$$n = 5.54\left(\frac{t_R}{W_{1/2}}\right)^2 \qquad N = 5.54\left(\frac{t'_R}{W_{1/2}}\right)^2$$

$$h = \frac{L}{n} \qquad H = \frac{L}{N}$$

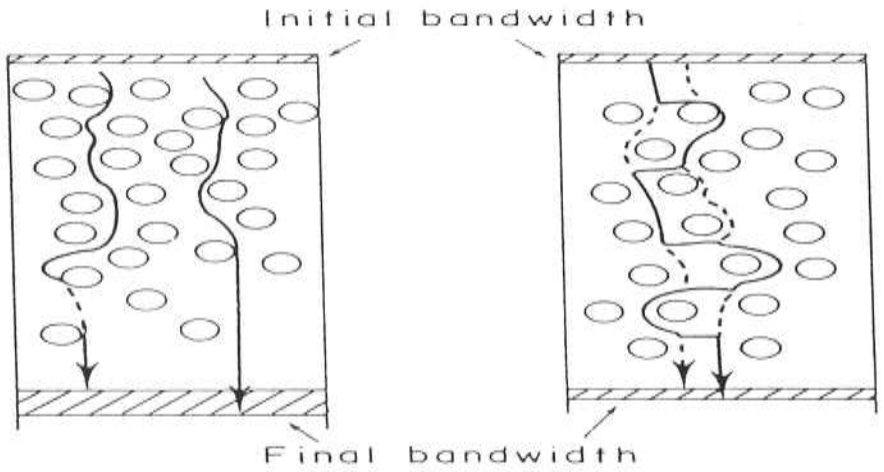
$$N = n\left(\frac{k'}{k'+1}\right)^2$$



Column Dispersion Mechanisms

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

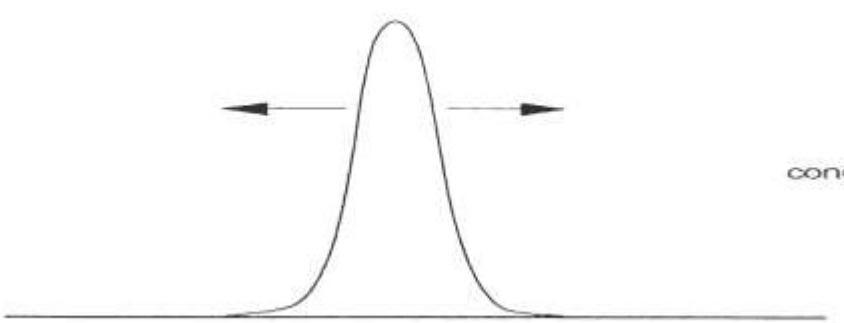
A term: Multiple path effect (Eddy diffusion)

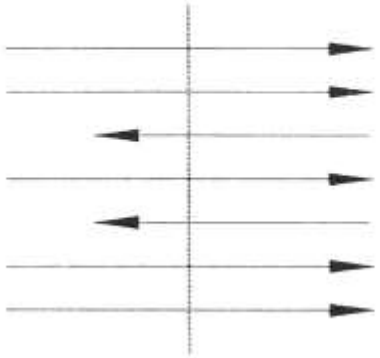


$$A = 2\lambda d_p$$

λ = a packing constant
(0.5 for well packed columns)
d_p = particle diameter

B term: Longitudinal (molecular) diffusion





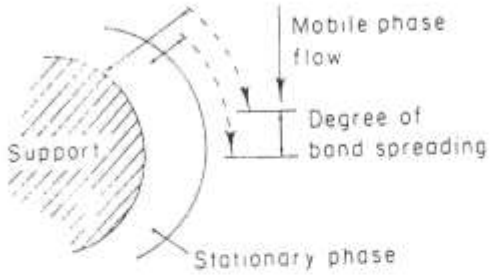
$$B = 2\gamma D_M$$

γ = obstruction factor; for packed columns typical values are 0.6-0.8
for capillary columns 1.0

D_M = diffusion coefficient; in liquids ~ 10^{-5} cm^2 s^{-1}
in gases ~ 10^{-1} cm^2 s^{-1}

C term: Mass transfer

C_s term: Stationary phase mass transfer



adsorption $$2t_d \frac{k'}{(1+k')^2}$$

partition $$\frac{d_f^2}{D_s} \frac{k'}{(1+k')^2}$$

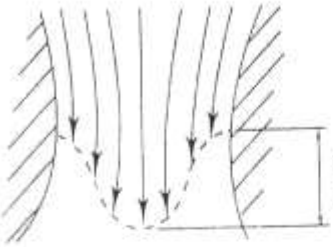
k' : capacity factor

t_d : mean desorption time (mean time that a solute particle remains attached to the surface of the stationary phase)

d_f : liquid film thickness

D_s: rate of diffusion of solute particle in liquid stationary phase

C_m and C_{sm} terms: Mobile phase mass transfer



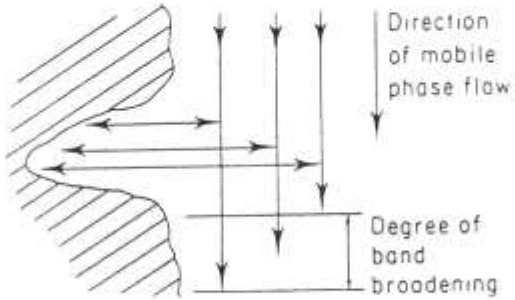
$$C_m = \Omega d_p^2 / D_M$$

'Moving' mobile phase mass transfer

Ω: is a function of the packing structure

d_p: particle diameter (packing material)

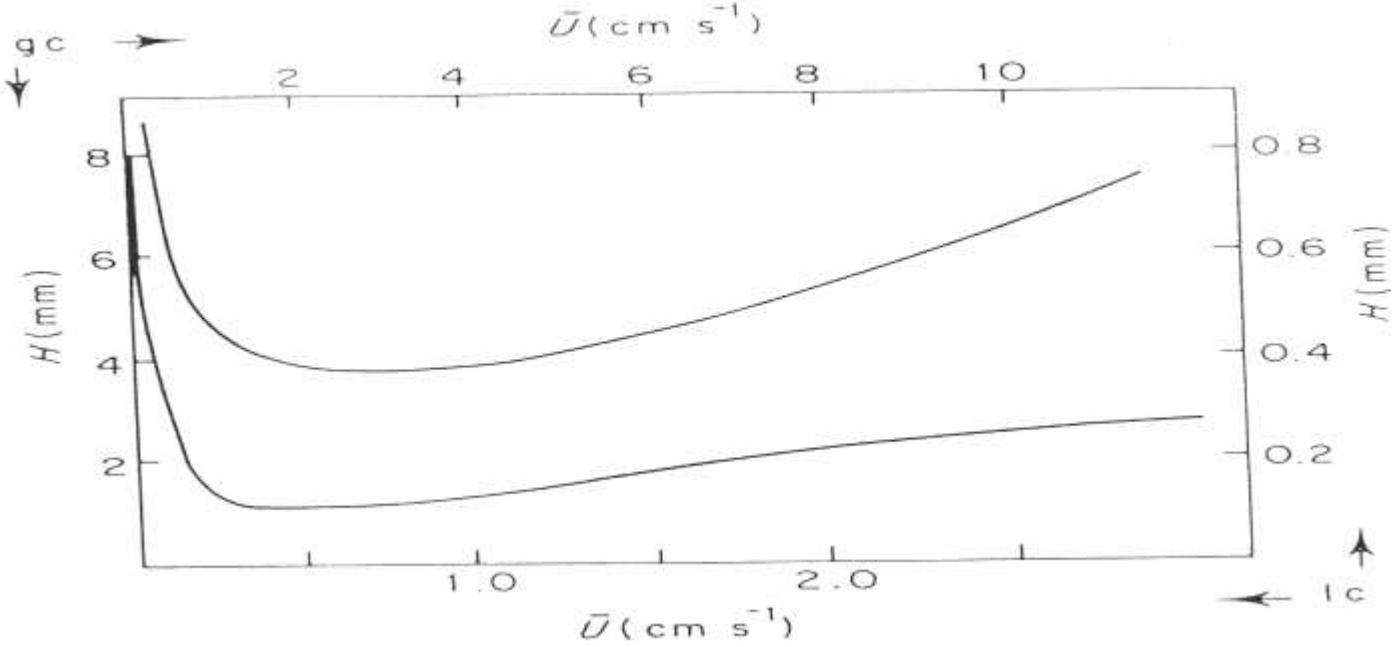
D_M: coefficient of diffusion of the solute species in the mobile phase



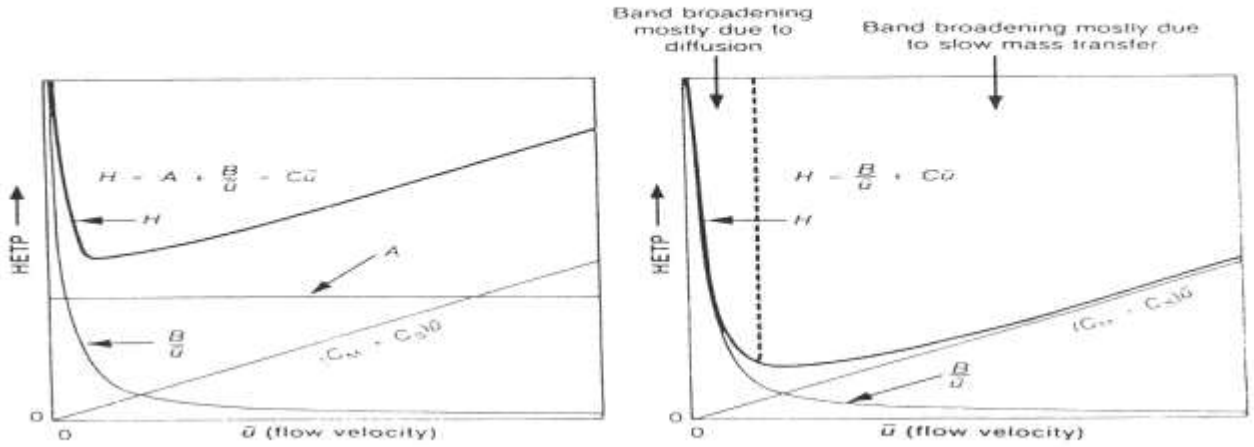
'Stagnant' mobile phase mass transfer

$$C_{sm} = \frac{(1-\phi+k')^2 d_p^2}{30(1-\phi)(1+k')^2 \gamma D_M}$$

ϕ: fraction of the total mobile phase in the intraparticle space



Variation of plate height with linear velocity

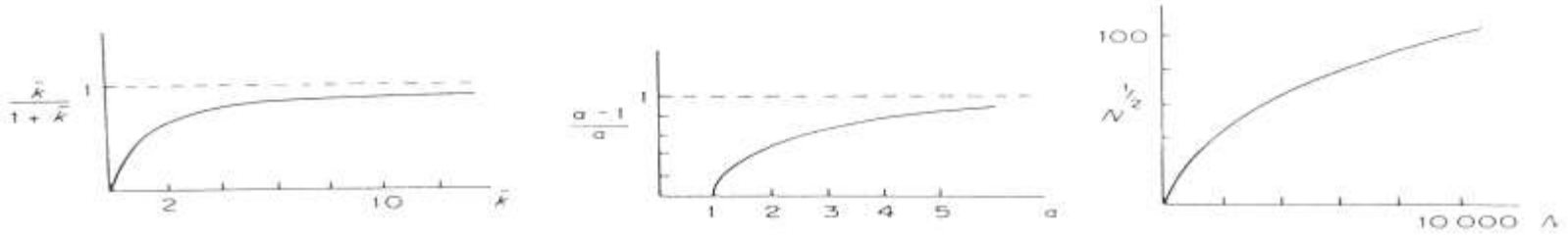


Resolution equation (Purnell Equation)

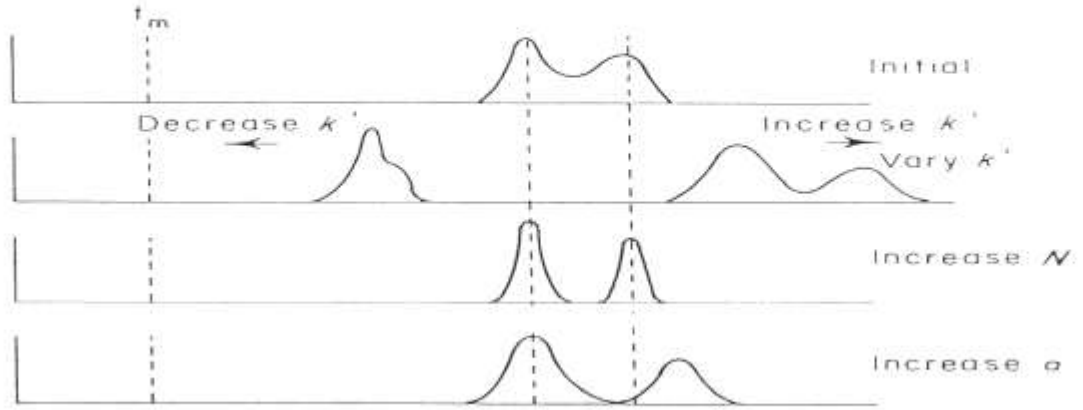
$$R_s = \frac{1}{2}\sqrt{n}\left(\frac{\alpha - 1}{\alpha + 1}\right)\left(\frac{\bar{k}'}{1 + \bar{k}'}\right)$$

Special case for two peaks eluting close together:

$$R_s = \frac{1}{4}\sqrt{n_2}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2'}{1 + k_2'}\right)$$



Effect of $\bar{k}$, α and N on resolution



Effect on R_s of changes in k' N and α

Peak Assymetry Factor