Analytical Chemistry

Postgraduate Students

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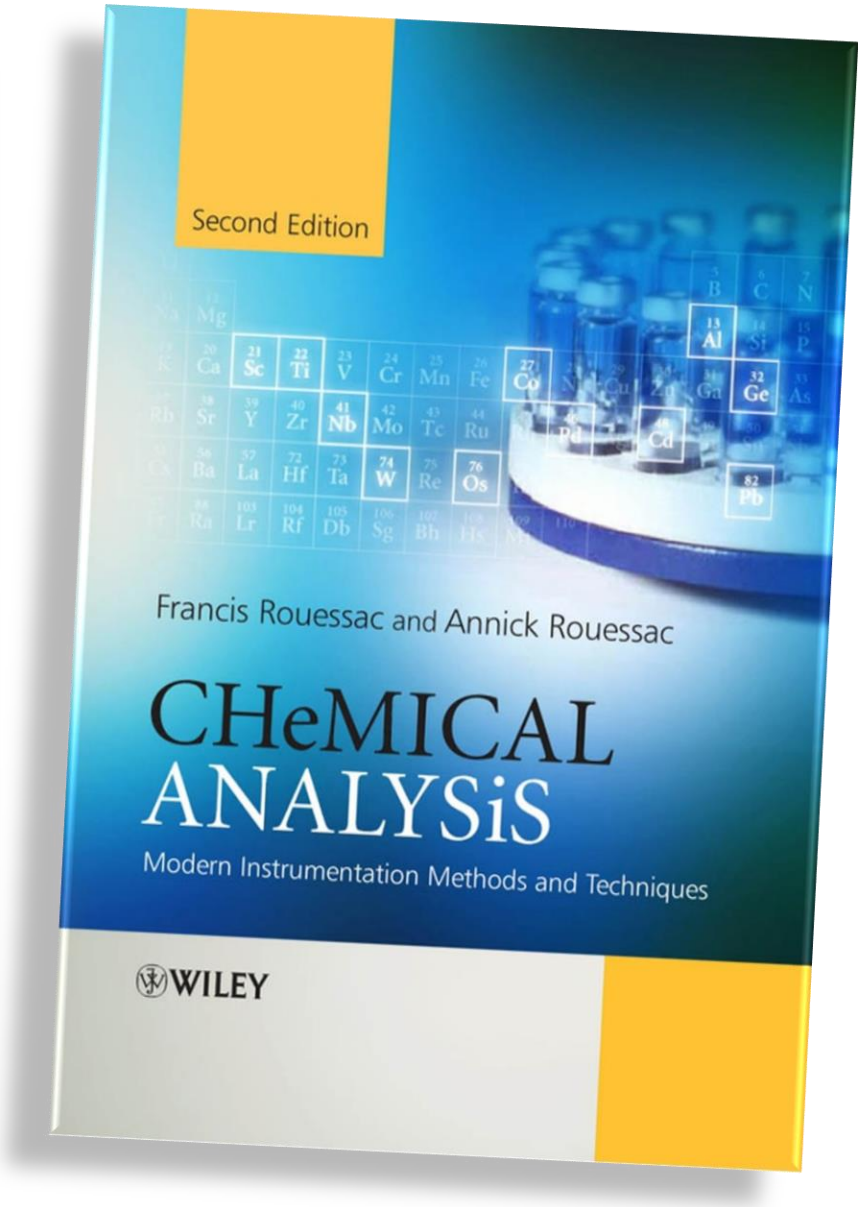
Ref//

- F. Rouessac and A. Rouessac, *Chemical Analysis, Modern Instrumentation Methods and Techniques*. *John Wiley & Sons, Ltd*, West Sussex, 2007.

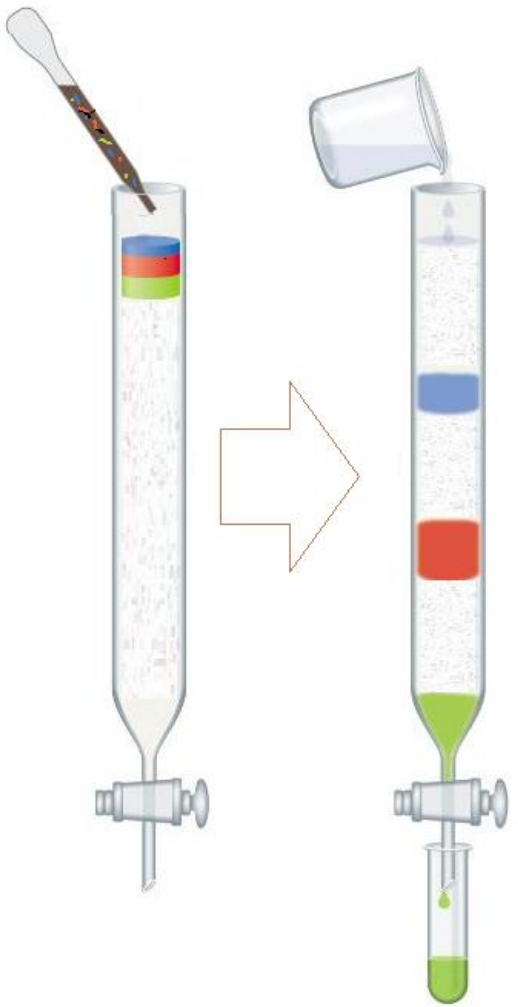


General aspects of chromatography

Chromatography, is a powerful tool for separating a gaseous or liquid state mixtures to their components.

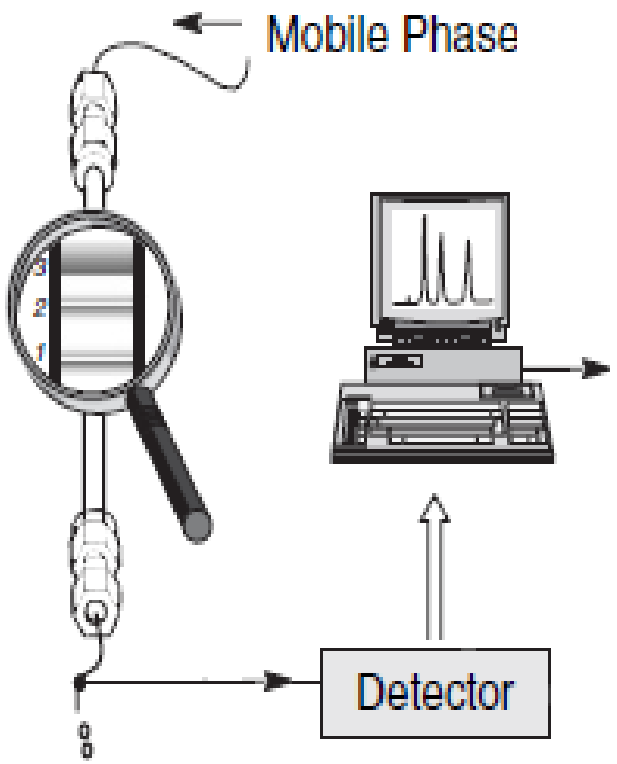
Principle, is based on the partitioning of a mixture components of interest between two immiscible phases, i.e. mobile and stationary phases. Since each component of a sample has different solubility in each phase. The differential migration of compounds leads to their separation.

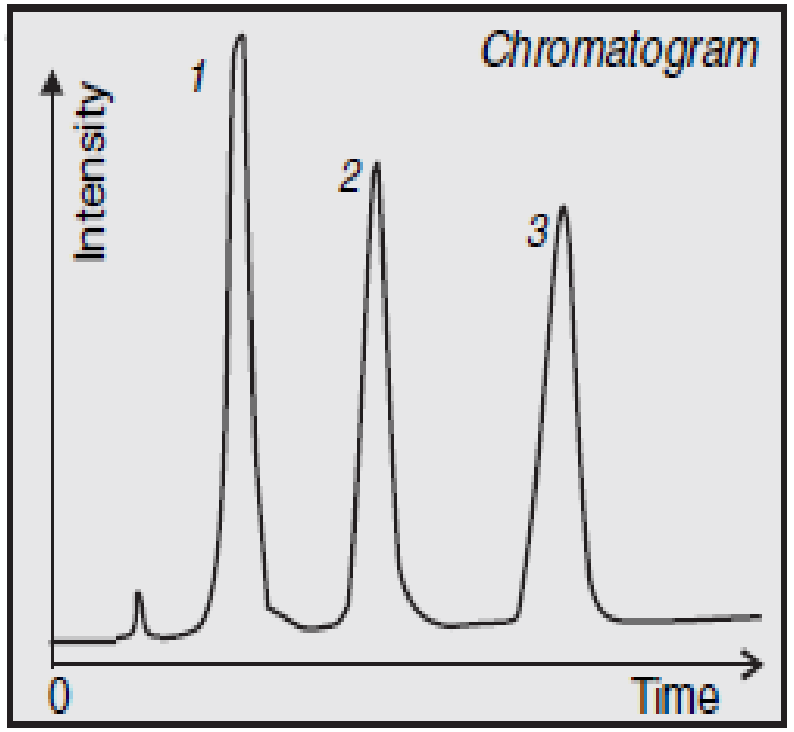
To identify a compound by chromatography, a solution of it must run on a certain analytical conditions to determine its ***retention time*** to be compared next with the ***chromatogram*** of the unknown.



The chromatogram

A **chromatogram** is the representation of the variation, with time (rarely volume), of the amount of an analyte. The separation is complete when the chromatogram shows as many chromatographic peaks as there are components in the mixture to be analyzed.

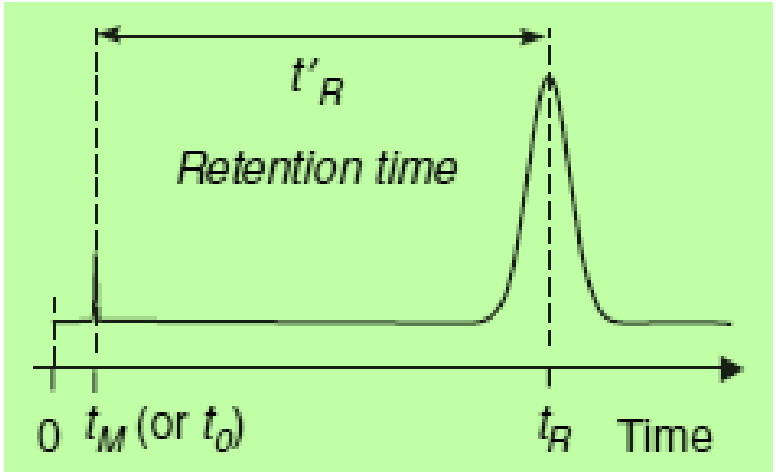




The chromatogram

If the **signal** sent by the sensor varies linearly with the ***concentration*** of a compound, then the same variation will occur for the area under the corresponding peak on the chromatogram.

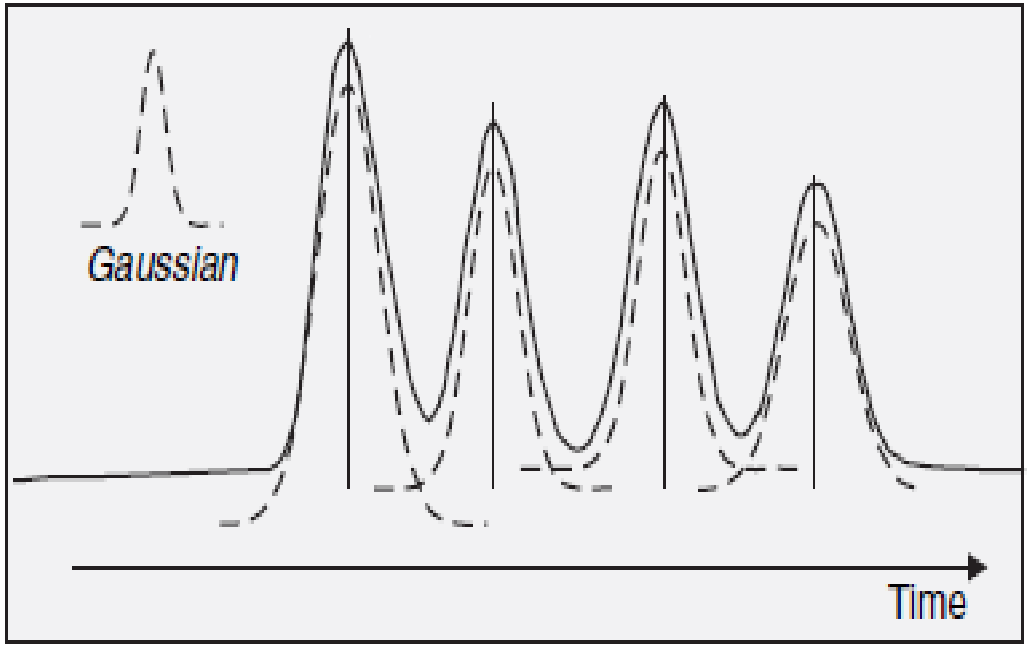
This is a basic condition to perform quantitative analysis from a chromatogram.



t_R	*retention time*	
t_0	*hold-up* or *dead time*	
t'_R	*adjusted retention time*	$[t'_R = t_R - t_0]$

Gaussian-shaped elution peaks

On a chromatogram the perfect elution peak would have the same form as the graphical representation of the law of Normal distribution of random errors (Gaussian curve).



Comparaison between a true chromatrogram and normal Gaussian-shaped peaks

Gaussian-shaped elution peaks

μ retention time of the eluting peak

σ the standard deviation of the peak

σ^2 the *variance*

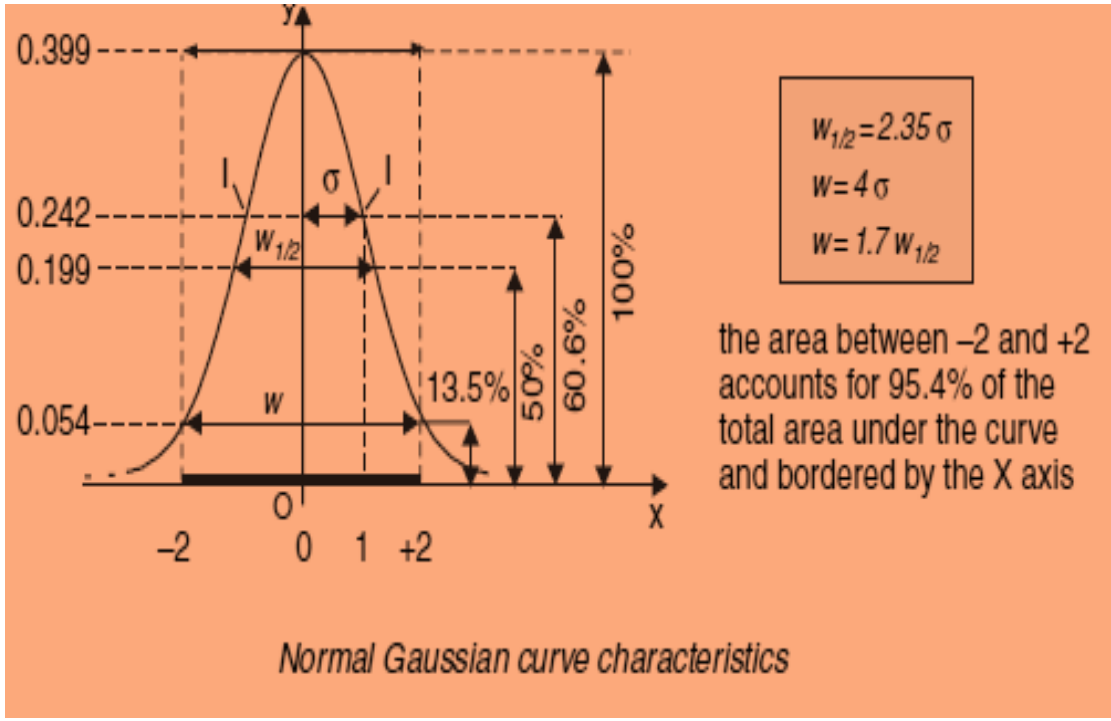
y the signal as a function of time x from the detector

$$y = \frac{1}{\sigma\sqrt{2\pi}} \cdot \exp\left[-\frac{(x-\mu)^2}{2\sigma^2}\right] \quad \textit{or} \quad y = \frac{1}{\sqrt{2\pi}} \cdot \exp\left[-\frac{x^2}{2}\right]$$

The width of the curve at the inflection points is equal to 2σ, $(\sigma = 1)$.

$w_{1/2}$ the width of the peak at half-height $(w_{1/2} = 2.35\sigma)$

w width of the peak 'at the base' and is measured at 13.5 per cent of the height , $w = 4\sigma$ by definition.



$w_{1/2} = 2.35\,\sigma$

$w = 4\,\sigma$

$w = 1.7\,w_{1/2}$

the area between –2 and +2 accounts for 95.4% of the total area under the curve and bordered by the X axis

Normal Gaussian curve characteristics

The plate theory

An important theory proposed to model chromatography and to explain the migration and separation of analytes in the column.

According to plate theory; a column of length L is sliced horizontally into N fictitious, small plate-like discs of same height H and numbered from 1 to n. For each of them, the concentration of the solute in the mobile phase is in equilibrium with the concentration of this solute in the stationary phase. At each new equilibrium, the solute has progressed through the column by a distance of one disc (or plate).

$$H = \frac{L}{N}$$

The height equivalent to a theoretical plate (HETP or H) will be given by equation:

Nernst partition coefficient (K)

The fundamental physico-chemical parameter of chromatography is the equilibrium constant K, termed the partition coefficient, quantifying the ratio of the concentrations of each compound within the two phases.

$$K = \frac{C_S}{C_M} = \frac{\text{Molar concentration of the solute in the stationary phase}}{\text{Molar concentration of the solute in the mobile phase}}$$

Values of K are very variable since they can be large (e.g. 1000), when the mobile phase is a gas or small (e.g. 2) when the two phases are in the condensed state. Each compound occupies only a limited space on the column, with a variable concentration in each place, therefore the true values of CM and CS vary in the column, but their ratio is constant.

Column efficiency

1. Theoretical efficiency (number of theoretical plates "N")

N is a relative parameter, since it depends upon both the solute chosen and the operational conditions adopted.

$$N = 16\frac{t_R^2}{w^2} \qquad \& \qquad N = 5.54\frac{t_R^2}{w_{1/2^2}}$$

Column efficiency

2. Effective plates number (real efficiency)

In order to compare the performances of columns of different design for a given compound – or to compare, in gas chromatography, the performances between a capillary column and a packed column – more realistic values are obtained by replacing the *total retention time* t_R, by the *adjusted retention time* t'_R

$$(t'_R = t_R - t_M)$$

$$N_{eff} = 16\frac{t'^2_R}{w^2} \quad \& \quad N_{eff} = 5.54\frac{t'^2_R}{w_{1/2}{}^2}$$

3. Height equivalent to a theoretical plate (HETP)

The *equivalent height of a theoretical plate* **H**, is calculated for reference compounds to permit a comparison of columns of different lengths. **H** does not behave as a constant, its value depends upon the compound chosen and upon the experimental conditions.

Retention parameters

1. **Retention times t_R**
2. **Retention volume (or elution volume) VR :** represents the volume of mobile phase necessary to enable an analyte migration throughout the column from the moment of entrance to the moment in which it leaves. If the flow rate F is constant, then:

$$V_R = t_R \cdot F$$

The volume of a peak, V_{peak} corresponds to that volume of the mobile phase in which the compound is diluted when leaving the column. It is defined by:

$$V_{peak} = w \cdot F$$

3. **Hold-up volume (or dead volume) V_M**

$$V_M = t_M \cdot F$$

Retention parameters

4. Retention (or capacity) factor k

When a compound of total mass m_T is introduced onto the column, it separates into two quantities: m_M, the mass in the mobile phase and m_S, the mass in the stationary phase. During the solute's migration down the column, these two quantities remain constant. Their ratio, called the *retention factor k*, is constant and independent of m_T:

$$k = \frac{m_S}{m_M} = \frac{C_S}{C_M} \cdot \frac{V_S}{V_M} = K\frac{V_S}{V_M}$$

The retention factor, also known as the *capacity factor k*, is a very important parameter in chromatography for defining column performances. Though it does not vary with the flow rate or the column length, k is it not a constant as it depends upon the experimental conditions. For this reason it is sometimes designated by k' rather than k alone.

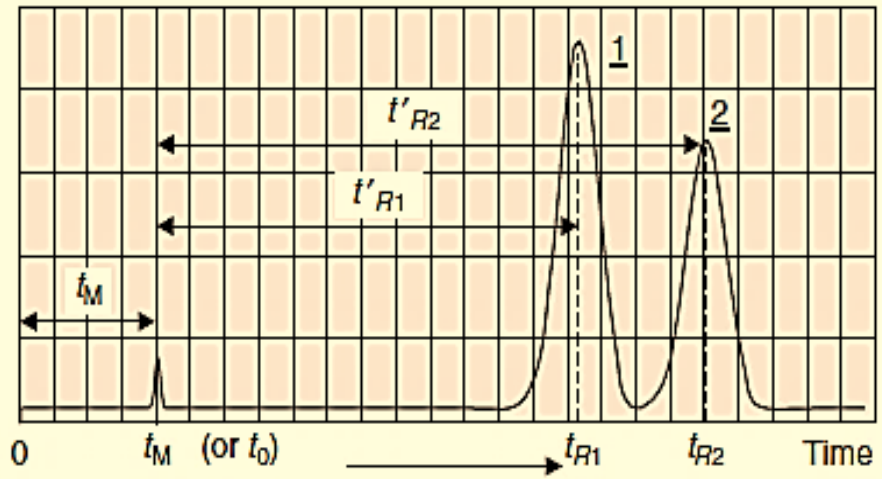
Retention parameters

4. Retention (or capacity) factor k

This parameter takes into account the ability, great or small, of the column to retain each compound. Ideally, **k** should be superior to one but less than five, otherwise the time of analysis is unduly elongated.

$$k = \frac{t'_R}{t_M} = \frac{t_R - t_M}{t_M} \quad \Rightarrow \quad t_R = t_M(1+k)$$

or $$V_R = V_M(1+k)$$



here,

$k_1 = \frac{t'_{R1}}{t_M}$ $k_1 = 3.07$

$k_2 = \frac{t'_{R2}}{t_M}$ $k_2 = 3.92$

$\alpha = \frac{t'_{R2}}{t'_{R1}}$ $\alpha = 1.27$

Retention factors and separation factor between two compounds. Each compound has its own retention factor. On this figure, the separation factor is around 1.3. The separation factor is also equal to the ratio of the two retention factors. α alone is not enough to determine whether the separation is really possible.

Chromatography

Separation (or selectivity) factor between two solutes

The separation factor α, (1.24) enables the comparison of two adjacent peaks 1 and 2 present in the same chromatogram above:

$$\alpha = \frac{t'_{R(2)}}{t'_{R(1)}} \qquad \text{or} \qquad \alpha = \frac{k_2}{k_1} = \frac{K_2}{K_1}$$

Resolution factor between two peaks "R"

To quantify the separation between two compounds, another measure is provided by the *resolution factor* **R**. Contrary to the selectivity factor which does not take into account peak widths:

$$R = 2\frac{t_{R(2)} - t_{R(1)}}{w_1 + w_2}$$

Retention parameters

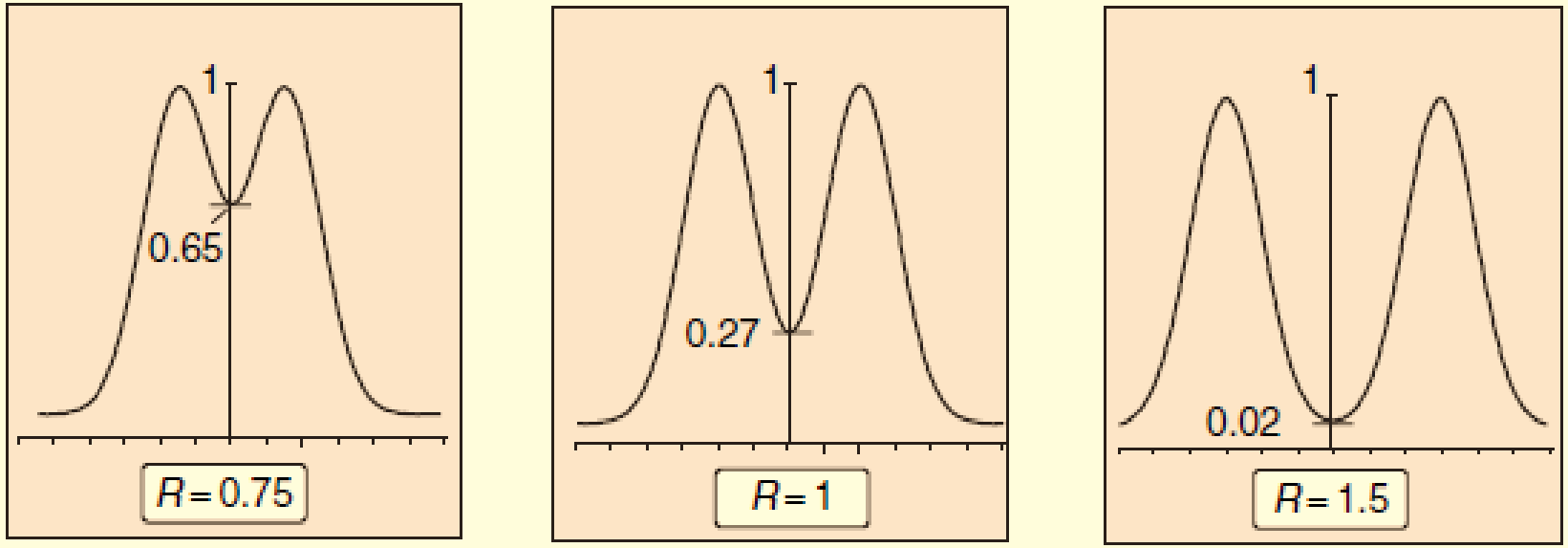


Figure *Resolution factor*. A simulation of chromatographic peaks using two identical Gaussian curves, slowly separating. The visual aspects corresponding to the values of R are indicated on the diagrams. From a value of R = 1.5 the peaks can be considered to be baseline resolved, the valley between them being around 2 per cent.

The rate theory of chromatography

You may notice that, in the plate theory, the velocity of the mobile phase in the column and solute diffusion are, perhaps surprisingly, never taken into account.

Rate theory is a more realistic description of the processes at work inside a column which takes account of the time taken for the solute to equilibrate between the two phases. It is the dynamics of the separation process which is concerned. The first kinetic equation *for packed columns in gas phase chromatography* was proposed by Van Deemter.

The rate theory of chromatography

Van Deemter's equation

It links the plate high **H** to the average linear velocity of the mobile phase $\bar{u}$ in the column:

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

If **H** is expressed in cm, **A** will also be in cm, **B** in cm^2/s and **C** in s (where velocity is measured in cm/s).

This equation reveals that there exists an *optimal flow rate* for each column, corresponding to the minimum of H. The loss in efficiency as the flow rate increases is obvious.

The rate theory of chromatography

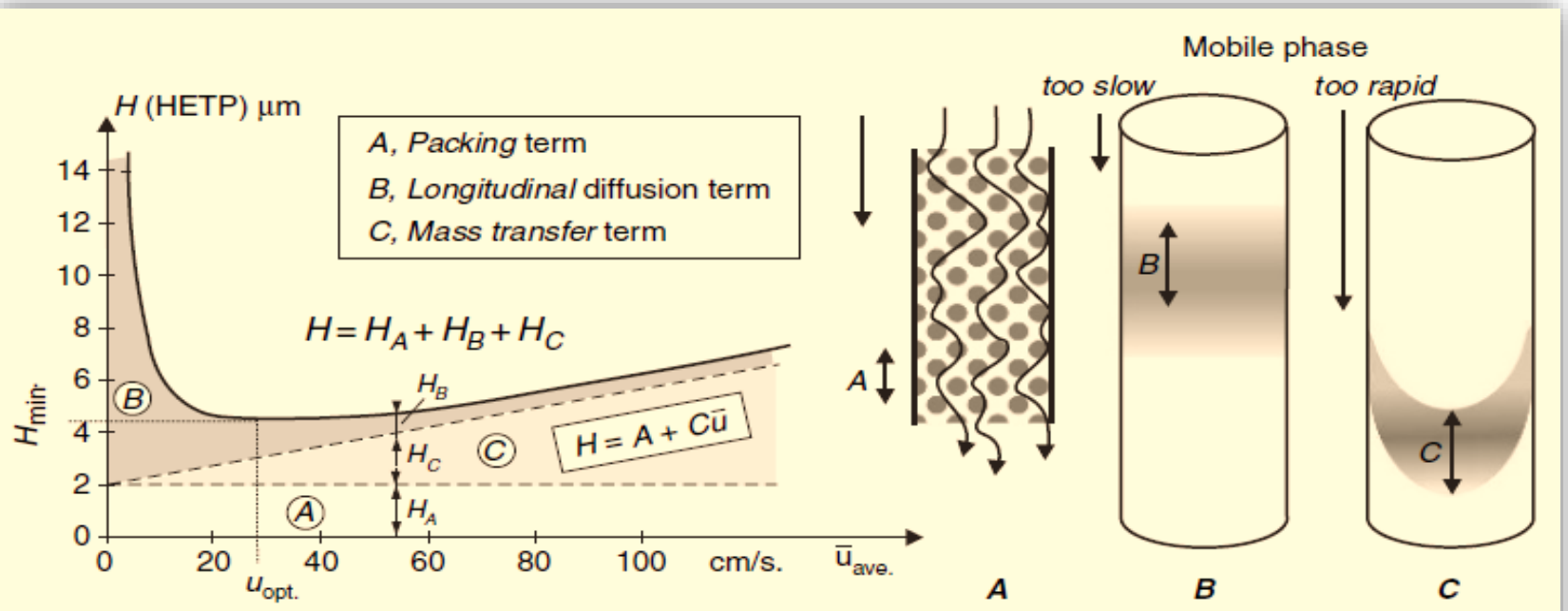


Figure *Van Deemter's curve in gas chromatography with the domains of parameters A, B and C indicated.* There exists an equation similar to that of Van Deemter that considers temperature: H = A + B/T + CT.

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

The curve that represents the Van Deemter equation is a hyperbola which goes through a minimum (H_{min}) when:

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

The rate theory of chromatography

Packing related term $A = 2\lambda . d_p$

Term **A** is related to the flow of the mobile phase passing through the stationary phase. The size of the particles (diameter $\mathbf{d_p}$), their dimensional distribution and the uniformity of the packing (factor characteristic of packing **λ**) can all be the origin of flow paths of different length which cause broadening of the solute band and improper exchanges between the two phases. This results in turbulent or ***Eddy diffusion***. For a given column, nothing can be done to reduce the **A** term.

Gas (mobile phase) term $B = 2\gamma D_G$

Term **B**, which can be expressed from D_G, the *diffusion coefficient* of the analyte in the gas phase and, the above packing factor, is related to the **longitudinal molecular diffusion** in the column. It is especially important when the mobile phase is a gas.

The rate theory of chromatography

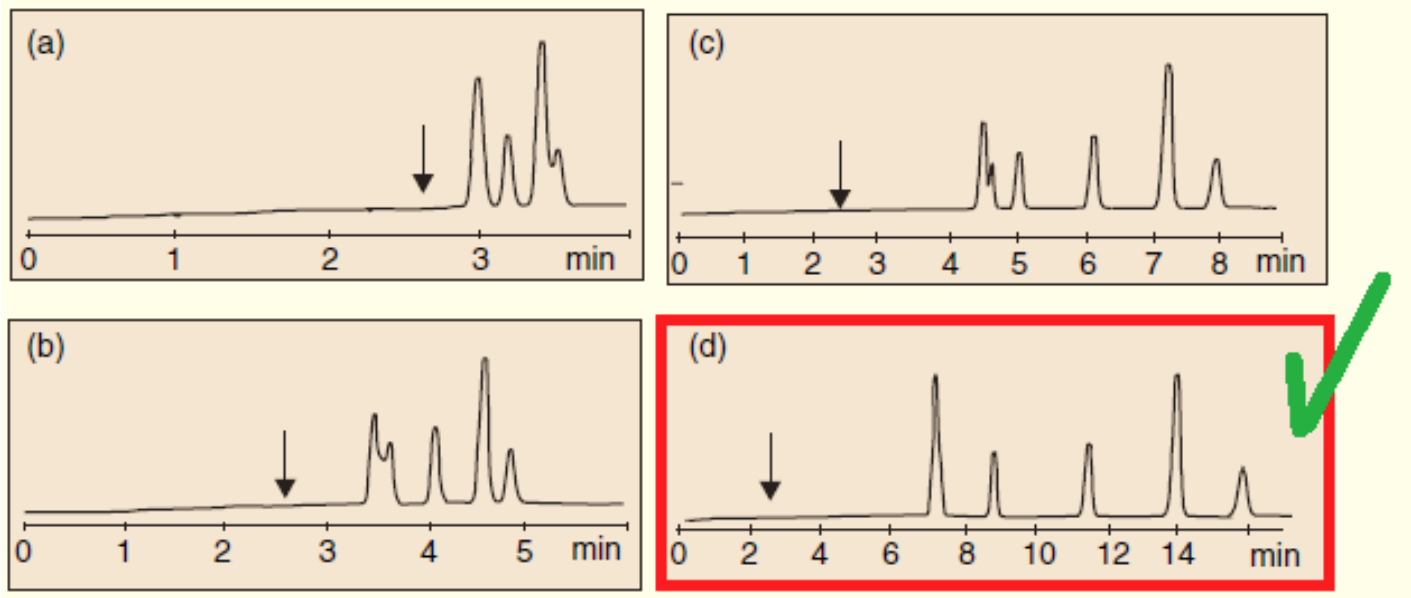
Liquid (stationary phase) term $C = C_G + C_L$

Term C, which is related to the ***resistance to mass transfer*** of the solute between the two phases, becomes dominant when the flow rate is too high for an equilibrium to be attained.
Local turbulence within the mobile phase and concentration gradients slow the equilibrium process ($C_S \Leftrightarrow C_M$). The parameter C_G is dependent upon the diffusion coefficient of the solute in a

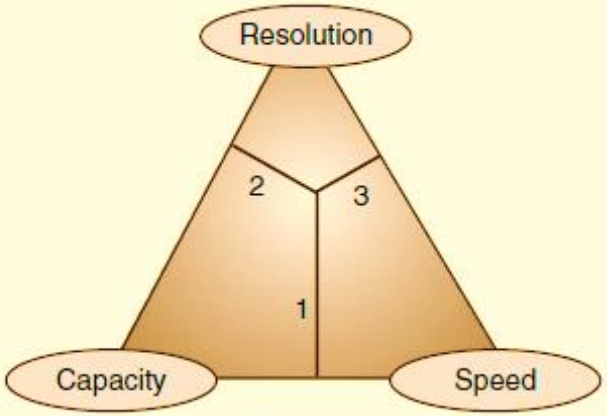
gaseous mobile phase, while the term C_L depends upon the diffusion coefficient
in a liquid stationary phase. Viscous stationary phases have larger C terms.

Optimization of a chromatographic analysis

The ***resolution*** and the ***elution time*** are the two most important dependent variables to consider. In all optimizations, the goal is to achieve a sufficiently complete separation of the compounds of interest in the minimum time.



Note: *interesting to separate a certain compound (focus on elution time) OR to fully separate the sample components (full resolution is critical).*



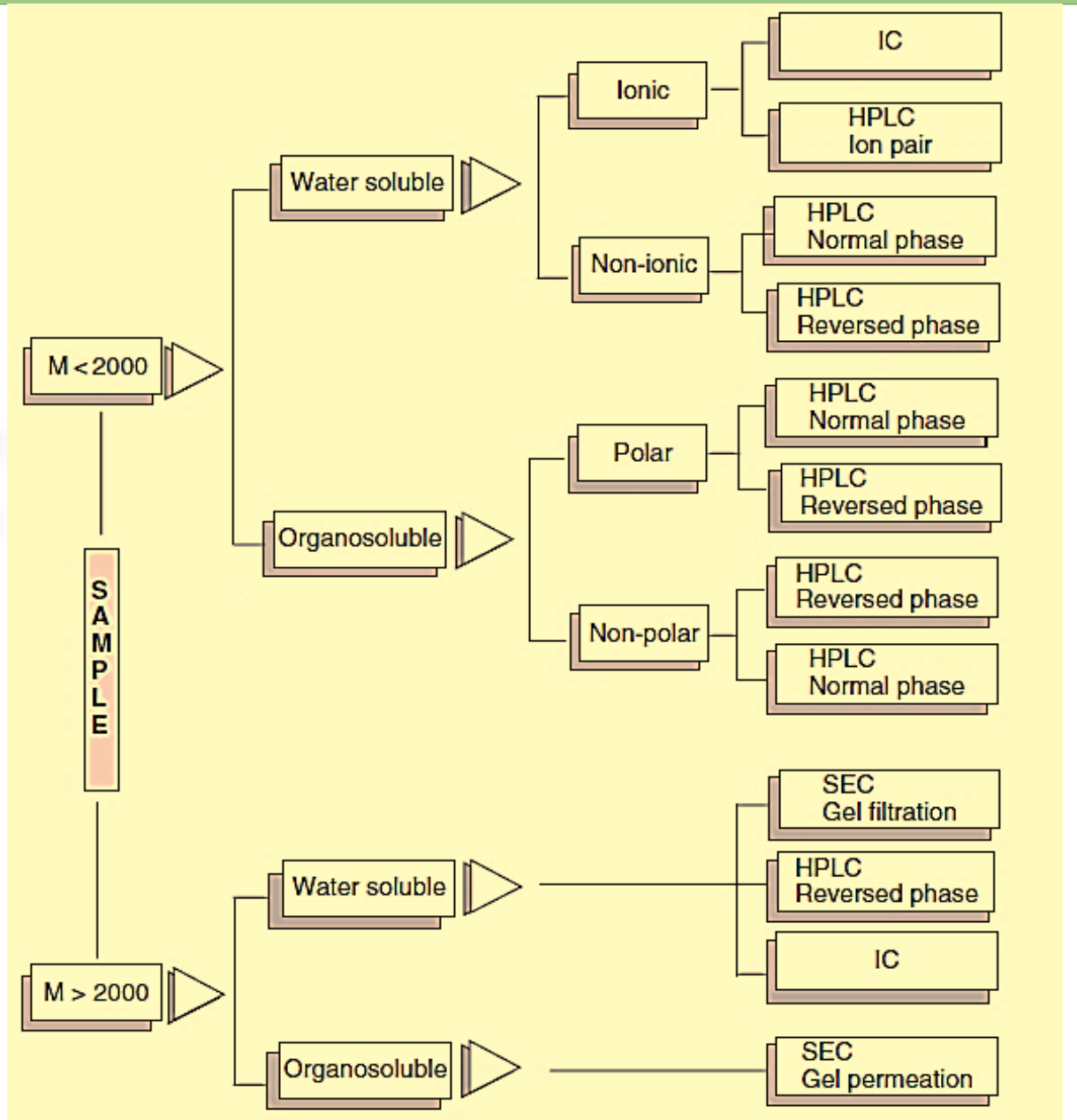
The chromatographer's triangle. The shaded areas indicate the domain corresponding to analytical chromatography based principally upon the five parameters K, N, k, α and R.

Classification of chromatographic techniques

1. **Liquid phase chromatography (LC)**

   1. ***Liquid/solid chromatography (or adsorption chromatography)***
      The physico-chemical parameter involved here is the *adsorption coefficient*.

   2. ***Ion chromatography (IC)***
      The mobile phase here is a buffer while the solid stationary phase has a surface with ionic sites. These phases allow the exchange of their mobile counter ion with ions of the same charge present in the sample. This type of separation relies on *ionic distribution coefficients*.



Classification of chromatographic techniques

3. *Size exclusion chromatography (SEC)*

The stationary phase here is a material containing pores whose dimensions are selected as a function of the size of the species to be separated.

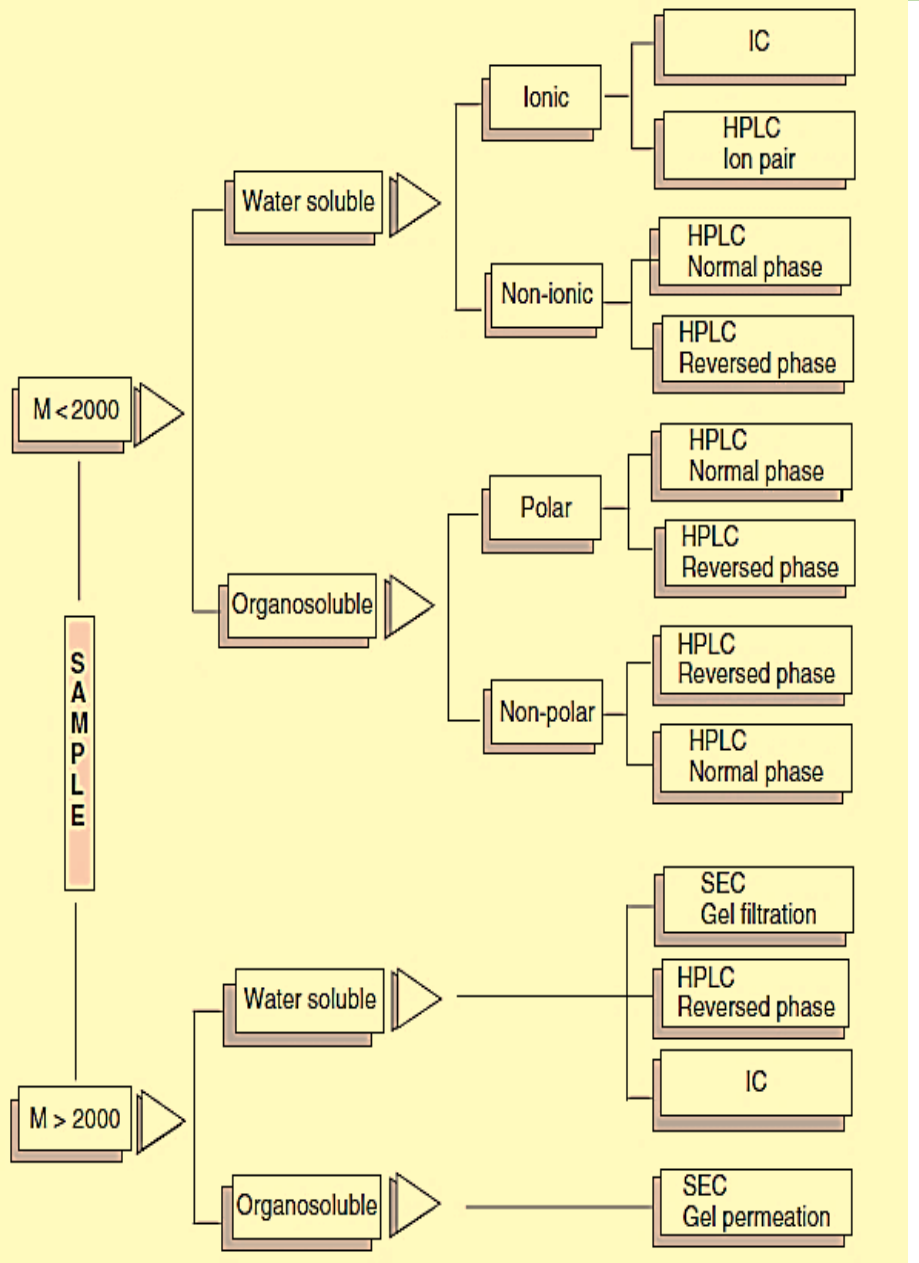
The usage of selective permeability leading to its name, *gel filtration* or *gel permeation* depending on the mobile phase, which is either aqueous or organic. For this technique, the distribution coefficient is called the *diffusion coefficient*.

4. *Liquid/liquid chromatography (or partition chromatography, LLC)*

The stationary phase is an *immobilized* liquid upon an inert and porous material, which has only a mechanical role of support.

Liquid/bound phase chromatography

In order to immobilize the stationary phase (generally a liquid polymer), it is preferable to fix it by covalent bonding to a mechanical support. The quality of separation depends upon the *partition coefficient K* of the solute between the two phases (similar to liquid/liquid extraction).



Classification of chromatographic techniques

Gas phase chromatography (GC)

Can be sub-divided according to the nature of the phase components:

a) Gas/liquid/ chromatography (GLC)
b) Gas/solid chromatography (GSC)

Supercritical fluid chromatography (SFC)

Here the mobile phase is a fluid in its supercritical state, such as carbon dioxide at about 50 °C and at more than 150 bar (15 MPa). The stationary phase can be a liquid or a solid. This technique combines the advantages of those discussed above: liquid/liquid and gas/liquid chromatography.

Problems at the end of the chapter//