INTRODUCTION TO CHROMATOGRAPHY

ERNST KENNDLER

Institute for Analytical Chemistry, University of Vienna

Ernst.Kenndler@univie.ac.at

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Version 19/01/2004

1 THEORY OF CHROMATOGRAPHY

Separation of two sample components in chromatography is based on their different distribution between two non-miscible phases. The one, the stationary phase, a liquid or solid, is fixed in the system. The other, the mobile phase, a fluid, is streaming through the chromatographic system. In gas chromatography the mobile phase is a gas, in liquid chromatography it is a liquid.
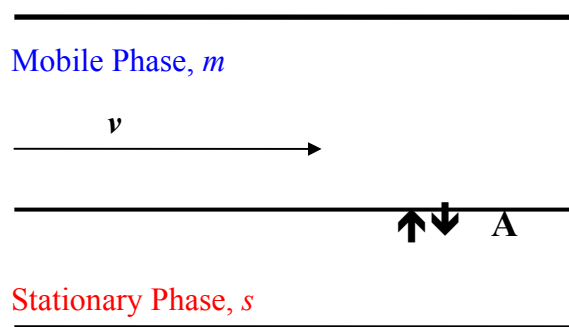
Mobile Phase, *m*

v

A

Stationary Phase, *s*

Figure 1 Schematic presentation of a chromatographic system with partition of analyte A between the phases; v mobile phase velocity.

The molecules of the analytes are distributed between the mobile and the stationary phase. When present in the stationary phase, they are retained, and are not moving through the system. In contrast, they migrate with the velocity, *v*, of the mobile phase when being there. Due to the different distribution of the particular analytes the mean residence time in the stationary phase differs, too, resulting in a different net migration velocity (see Figure 2). This is the principle of chromatographic separation.

Principle of chromatographic separation: Different distribution of the analytes between mobile and stationary phase results in different migration velocities

The position of the distribution equilibrium determines the migration velocity. It reflects the intermolecular interactions of the analyte with the stationary and the mobile phase. If only this process is considered, separation of the analytes as schematically shown in Figure 2 would be the result.
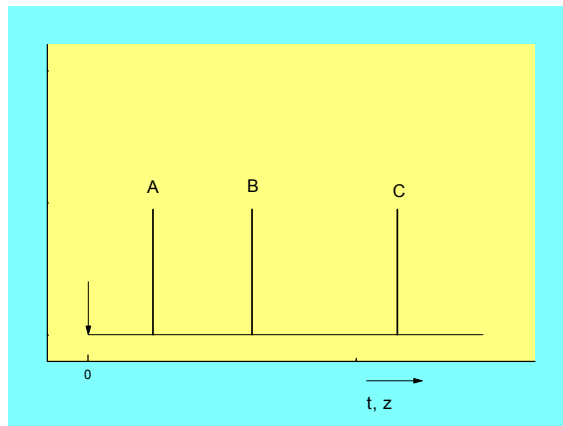
Figure 2 Separation of analytes A, B and C in time, t, or space, z, respectively, The analytes are inserted initially in a narrow zone. No zone broadening is taken into account.

However, during migration of the analytes through the separation capillary broadening of the original sample zone takes place. This broadening is greater the longer the migration distance is. Two effects are therefore counteracting in the chromatographic system: the different migration is the base of separability by introducing separation selectivity. In contrast, peak broadening effects lead to a potential overlap of the peaks migrating with different velocity. They occur in parallel with a dilution of the initial sample zone with the mobile phase.
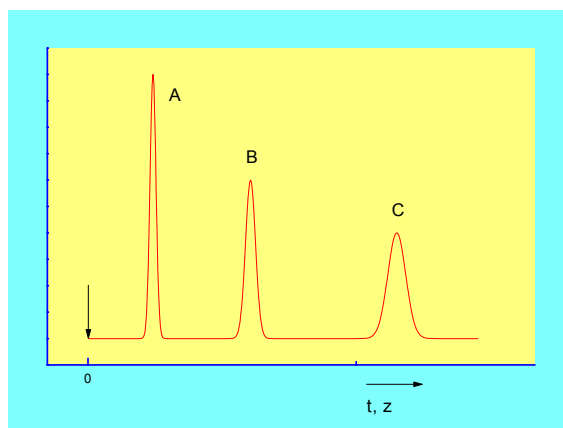

Figure 3 Depiction of a chromatogram of analytes A, B and C, in the time (t) or space (z) ordinate, respectively.

Partitioning of analyte molecules between mobile and stationary phase is a stochastic process. Dispersion occurs around a mean value. Dispersion is more pronounced as more

partitioning steps occur: thus it increases with increasing migration distance. The result is the formation of broadened analyte peaks, leading to a chromatogram as schematically depicted in Figure. 3.

An appropriate theory of chromatography must be able to describe quantitatively the two counteracting phenomena: (i) the different, specific migration velocity and (ii) peak dispersion. It should finally enable to express the extent of peak separation by a characteristic number, the chromatographic resolution.

From the methods used in practise (see Table about the systematic), the following discussion will concentrate on

linear analytical elution chromatography

SYSTEMATIC OF CHROMATOGRAPHY

state of phases	gas / liquid	GLC	
	gas / solid	GSC	
	liquid / liquid	LLC	
	liquid / solid	LSC	
mechanism	Partition, absorption adsorption	2 bulk phases 1 bulk phase, 1 surface	
geometry	column	packed open tubular	
	planar	thin layer paper	
amount of separand	analytical preparative		
composition of mobile phase	elution displacement	linear non-linear	gradient isocratic

1.1 Migration

1.1.1 Migration velocity, capacity or retention factor

We consider the concentration, c_i, of an analyte, i, in a small segment of the chromatographic system with length Δz, and cross section area A^s and A^m, respectively. This concentration varies with time.
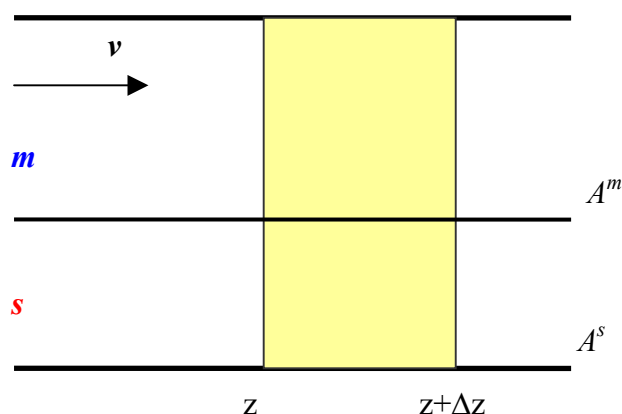

Figure 4 Schematic representation of a segment of the chromatographic system between z and z+Δz.

The mole fraction of analyte, i, being in average in the mobile phase, m, is given by

$$\frac{n_i^m}{n_i^m + n_i^s} \qquad (1)$$

where n_i is the mole number of i. Substitution of the mole number gives

$$\frac{1}{1+\frac{n_i^s}{n_i^m}} = \frac{1}{1+\frac{c_i^s}{c_i^m}\frac{V^s}{V^m}} = \frac{1}{1+k_i^{`}} \qquad (2)$$

$k^{`}$ is the mass distribution coefficient

$$k_i^{`} = \frac{n_i^s}{n_i^m} = \frac{c_i^s}{c_i^m}\frac{V^s}{V^m} = K_i\, q \qquad (3)$$

Here c is the molar concentration, V the phase volume, K_i is the Nernst concentration distribution constant, and q the phase ratio:

$$K_i = \frac{c_i^s}{c_i^m} \qquad q = \frac{V^s}{V^m} \tag{4}$$

It can be seen that k_i` depends on

➤ the unspecific phase ratio q, which is equal for all analytes

➤ the concentration distribution constant, K_i, that reflects the specific chemical interactions.

For the derivation of an expression for the migration velocity, u_i, of the separand i, we ask for the relative amount of analyte, i, which is in average in the mobile phase. This ratio is equal to the probability of one molecule being in average in the mobile phase, and is given by eq. 2, too. When present in the mobile phase the analyte molecule migrate with velocity, v. Consequently the fraction of analyte, i, being in average in the stationary phase, s, and thus the probability being there is

$$\frac{k_i^{'}}{1+k_i^{'}} \tag{5}$$

The migration velocity is therefore

$$u_i = \frac{1}{1+k_i^{'}} v + \frac{k_i^{'}}{1+k_i^{'}} 0 \tag{6}$$

or

$$u_i = \frac{v}{(1+k_i^{'})} \tag{7}$$

It can be seen that u_i depends on the mean linear velocity, v, and on the mass distribution coefficient, k_i`.

The mass distribution coefficient, k_i`, is of central importance for the description of the chromatographic migration; nevertheless, it determines the extent of peak dispersion as well. $k_i$` is named retention factor, capacity factor or capacity ratio. It was already mentioned that k_i` consists of an unspecific part, the phase ratio, that is equal for all analytes for a given chromatographic system. It consists further from the concentration distribution constant K_i, in which the specific chemical interactions between the analyte molecules and the stationary phase are reflected. As in gas chromatography (GC) the mobile phase is considered as an ideal gas (that is inert by definition) no chemical interactions occur here. Contrary to liquid chromatography (LC) only one dimension exists thus in GC to influence the migration velocity of the analytes in a specific way for optimisation of the chromatographic selectivity.

1.1.2   Planar chromatography

In planar chromatography the separation system is a layer. Sample components are separated by a solvent, which is moving through the system, typically by capillary forces. Therefore the mobile phase velocity is not constant, but decreases with time. After a certain time the development of the chromatogram is stopped. This means that the migration time is equal for all components, but their migration distance, z_i, differs. The migration distance of a non-retained component is equal to that of the solvent front, z_0 (see Figure 5).
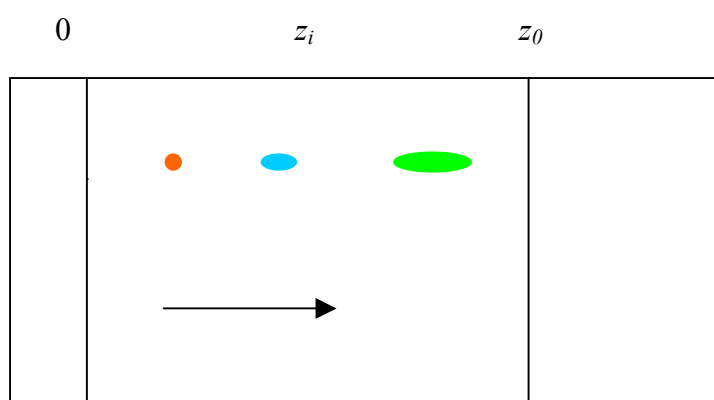

Figure 5 Scheme of a planar chromatogram with three analytes

The retention factor, R_F, relates the migration distance of an analyte with velocity u_i to the solvent front

$$R_{Fi} \equiv \frac{z_i}{z_0} \tag{8}$$

With

$$z_i = u_i t \qquad z_0 = vt \tag{9}$$

the R_F value is

$$R_{Fi} = \frac{1}{1 + k_i^{'}} \tag{10}$$

1.1.3   Retention time, t_{Ri}

The retention time is the time in which halve of the quantity of a solute, *i*, is eluted from the chromatographic system. With other words, it is identical with the position of the peak maximum in case of a Gaussian elution profile. It is determined by the length of the column, *L*, and the migration velocity of the solute

$$t_{Ri} = \frac{L}{u_i} = \frac{L(1 + k_i^{;})}{v} \tag{11}$$

with t_{R0} , the residence time of a non-retained component

$$\frac{L}{v} = t_{R0} \tag{12}$$

the dead time, which is the time the mobile phase needs to stream through the capillary column with an average linear velocity *v* measured over entire length, *L*. The retention time is given by

$$t_{Ri} = t_{Ro}(1 + k_i^{;}) \tag{13}$$

We can rewrite this equation as

$$t_{Ri} = t_{R0} + t_{R0}k_i^{;} \tag{14}$$

and can interpret it as following: the total retention time consists of two contributions

$$t_{Ri} = t_m + t_s$$

The one is t_m, which is identical with t_{R0}, the time the molecules are in mobile phase. This is equal for all components (and therefore non-specific). In the mobile phase the molecules exhibit the same velocity.

The second part is the solute-specific contribution on the total retention time, that part which determines the separation selectivity. It is the *k´*-fold dead-time ($t_{R0}.k´$). This contribution depends on K_i, and consequently on the chemical interactions between analyte and stationary liquid. Separation selectivity can thus be established due to the selection of the phase system, when differences in the partition constants are achieved.

Transformation of eq. 13 leads to an expression that enables the experimental determination of the capacity factor:

$$k_i^{;} = \frac{t_{R,i} - t_{R0}}{t_{R0}} \tag{15}$$

$k_i´$ can be calculated from the retention time of the analyte, and the residence time of a non-retained compound.

In contrast to LC the mobile phase in GC is a compressible medium, and the velocity of the mobile phase differs across the separation column: at the top of the column it is smaller than near the open end. It is therefore the mean linear velocity $<v>$, averaged over the column length, which is to be used in the equations given above.

1.2 Dispersion

1.2.1 Residence time distribution

During the movement of the analytes through the separation column processes occur that lead to a broadening of the sample zone. Empirically, and from the theory of chromatography it follows that zone broadening increases with increasing migration distance.

The residence time distribution function describes the concentration of an analyte in the mobile phase, c_i^m, as function of time and space, i.e. either in the time domain or in the space domain:

$$c_i^m = f(t,z) \tag{16}$$

The chromatographic column acts (under certain boundary- and limiting conditions) as Gaussian operator on the concentration distribution. Under the conditions that the sample is initially brought into the system in an infinitely narrow zone (a so-called δ- or Dirac function), and that the peak width is negligible compared to the migration distance ($\sigma_t \ll t$) the resulting concentration partition function is given by a normal distribution.
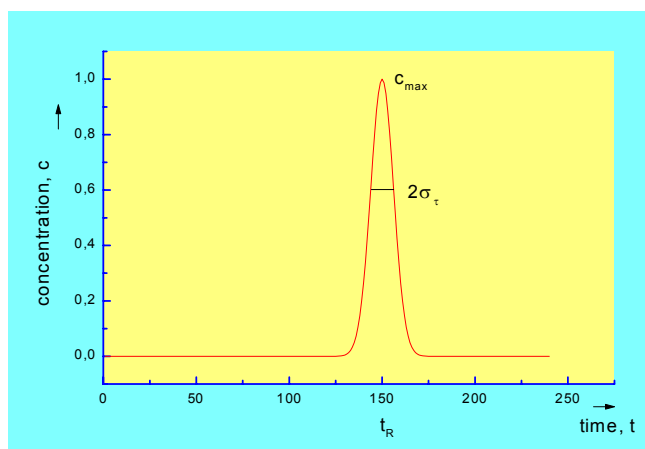

Figure 6 Gaussian concentration distribution function of an analyte with retention time, t_R

The resulting function is depicted in Figure 6 in the time domain. It gives the concentration of the component as function of time at a given position in the column (reasonably that of the detector after migration distance, L). The spatial distribution function (the concentration at a certain time as a function of the spatial coordinate) is of minor importance in column chromatography. It has relevance in planar chromatography.

The Gaussian bell curve is determined by three characteristic parameters:

$\Rightarrow$ the position of the concentration maximum, the retention time, t_{Ri}

$\Rightarrow$ the height in the maximum, $c^m_{max,i}$, the highest concentration of the analyte in the mobile phase

$\Rightarrow$ the width of the function, expressed e.g. by the standard deviation (here in the time domain), $\sigma_{t,i}$.

The Gaussian concentration partition function can be expressed as

$$c_i^m = c_{max,i}^m e^{-\frac{1}{2}\left(\frac{t_{Ri}-t}{\sigma_{t,i}}\right)^2} \tag{17}$$

The maximum concentration is given by

$$c_{max,i}^m = \frac{n_i}{\sqrt{2\pi}A^m\sigma_{t,i}(1+k_i^{'})} \tag{18}$$

A^m is the cross sectional area of the mobile phase.

It follows from eq. 17 that the standard deviation (as a measure for the peak width) can be used as a central variable to describe peak dispersion

1.2.2 Plate height, plate number

It is one of the central facts in chromatography that the spatial standard deviation, σ_z, increases with increasing migration distance. This increase is not proportional, but under-proportional related to the distance. In fact it is the variance, σ_z^2, the square of the standard deviation in the space domain, which is directly proportional to the length of migration, z

$$\sigma_z^2 = Hz \tag{19}$$

The factor of proportionality, H, is called plate height (height equivalent of a theoretical plate, HETP) and has the dimension of a length.

Note that the variance in the space domain, σ_z, and time domain, σ_t, respectively, are related to each other by the migration velocity:

$$\sigma_{t,i}^2 = \frac{\sigma_{z,i}^2}{u_i^2} = \frac{H_i L}{u_i^2} = \frac{H_i L}{L^2 / t_{Ri}^2} = \frac{H_i}{L} t_{Ri}^2 \tag{20}$$

The plate height, H, describes the extent of peak dispersion, caused by the mixing of the analyte with the mobile phase. The smaller peak broadening is (after a certain distance) the smaller is the value of H. It is the result of 4 processes that occur during the migration of the analyte zone through the separation column. Therefore the plate height is composed from 4 incremental contributions, which in sum determine peak dispersion:

$\Rightarrow$ H_{diff} describes the contribution from longitudinal diffusion

$\Rightarrow$ H_{conv} that from convective mixing

$\Rightarrow$ $H_{ex,m}$ that stemming from the kinetics of mass exchange from the mobile phase to the interface between mobile and stationary phase

$\Rightarrow$ $H_{ex,s}$ that from the kinetics of mass exchange from the stationary phase.

Consequently the total plate height is the sum of the four contributions:

$$H = H_{diff} + H_{conv} + H_{ex,m} + H_{ex,s} \tag{21}$$

It must be pointed out that the adequate measure for peak broadening which is relevant for peak separation is the plate number, N, rather than the plate height. It is given by

$$N = \frac{L}{H} \tag{22}$$

In practice the plate number is determined from the peak in the chromatogram by

$$N = \left(\frac{t_{Ri}}{\sigma_{t,i}} \right)^2 = \left(\frac{L}{\sigma_{z,i}} \right)^2 \tag{23}$$

1.2.3 Packed columns

It was pointed out above that peak broadening is the result of 4 processes, which incrementally contribute to the total plate height. The total plate height is a function of the following experimental parameters and variables:

$$H = f\left(v, D_{mi}, D_{si}, k_i', d_p, d_c, d_f, \varphi \right) \tag{24}$$

which are listed in the following Table:

v	average velocity of mobile phase
D_{mi}	diffusion coefficient in mobile phase
D_{si}	diffusion coefficient in stationary phase
k_i'	capacity or retention factor
d_p	particle diameter of packing
d_c	capillary diameter
d_f	film thickness of stationary phase
φ	geometric factor for irregularity of packing

The particular contributions are described in more detail in the following.

H_{diff}

The sample zone forms a concentration gradient in z direction, which is the cause of a diffusional mass transport in the longitudinal direction according to 1st Fick`s law:
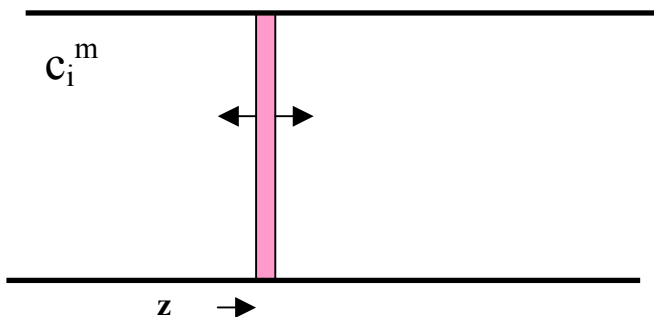
$$\frac{dc}{dt} = -D\frac{dc}{dz} \tag{25}$$

Figure 7 Longitudinal diffusion of sample zone in mobile phase.

This diffusional mass transport is independent of partition and flow, it depends only on the diffusion coefficient, D_{mi}, and the time available for diffusion . This time is proportional 1/v.

H_{conv}

In a packed bed the channels between the particles have different size and direction. Therefore both, the magnitude and direction of the local flow vector, are inhomogene, and the solute molecules migrate with different velocity at different positions in the column. The resulting peak broadening is independent of partition, and depends on v, d_p, D_{mi}. The plate height increases with increasing mobile phase velocity. Note that this effect of peak broadening is diminished by transversal diffusion.
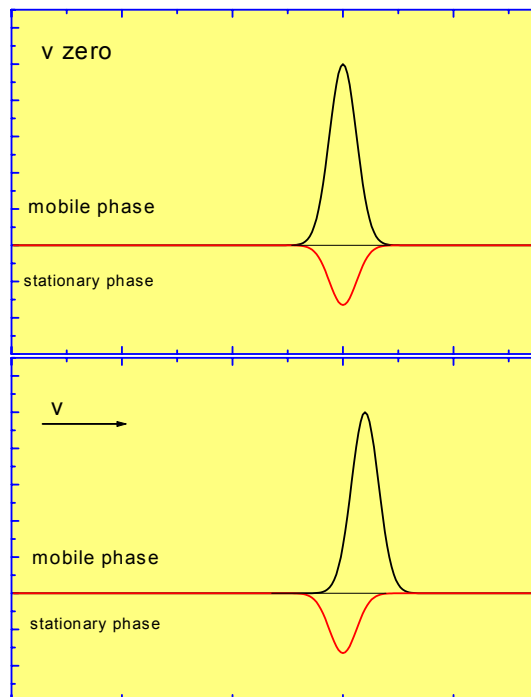
$H_{ex,m}$, $H_{ex,s}$

Figure 8 Schematic presentation of the sample zone in the mobile and the stationary phase.

Peak broadening in the flowing system occurs because there is time needed for the transport of the solute molecules from the mobile and the stationary phase, respectively, to the interface. For the (unrealistic) case that the fluid phase is not moving, partitioning between the

two phases has sufficient time to reach the stationary state. Therefore no additional peak broadening due to the finite kinetics of mass exchange would occur. The situation changes, however, in the practical case when the fluid phase is moved, because then at the front of the peak there is an excess of solute molecules in the mobile phase, at the rear side of the peak an excess is in the stationary phase, and the resulting peak broadens.

The kinetics of mass transfer is dependent on v, $k_i\grave{}$, D_{mi}, D_{si}, d_p, φ. It increases with mobile phase velocity.

In summary in the following Table the influence of increasing values of the parameters v, D_{mi}, D_{si}, d_p on the particular plate height contributions are listed

$\downarrow$ = H decreases, $\uparrow$ = H increases

	v	D_{mi}	D_{si}	d_p
H_{diff}	$\downarrow$	$\uparrow$	-	-
H_{conv}	$\uparrow$	$\downarrow$	-	$\uparrow$
$H_{ex,m}$	$\uparrow$	$\downarrow$	-	$\uparrow$
$H_{ex,s}$	$\uparrow$	-	$\downarrow$	$\uparrow$

1.2.4   Plate height vs. mobile phase velocity (van Deemter plot)

One of the most important experimental variables is the mobile phase velocity, because it enables to adjust separation efficiency. The dependence of the plate height on this velocity is described by the van Deemter equation, which reads in a simplified form as

$$H = A + B/v + Cv \tag{26}$$

The parameter A includes so-called eddy dispersion for packed columns, B stems from longitudinal diffusion and C from mass exchange resistance.

In practice in liquid and gas chromatography, respectively, a dependence as shown in Figure 9 is observed
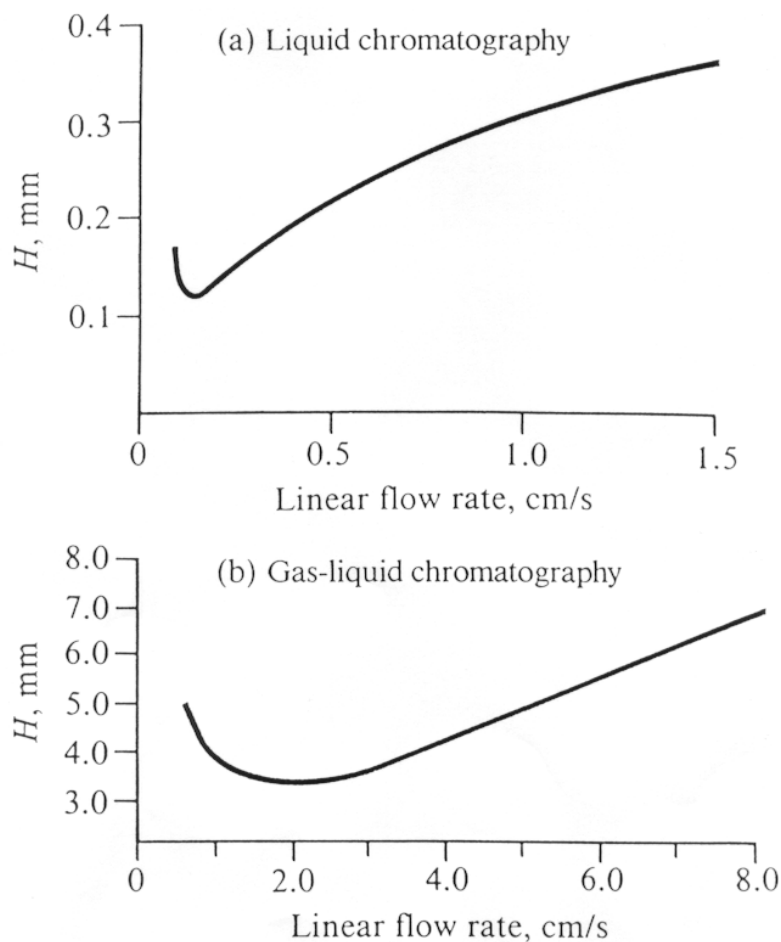
Figure 9 van Deemter plots for GC and LC: plate height, H, vs. mobile phase flow rate

It can be seen that a singular velocity exists where the plate height has a minimum. Left from the minimum, in the area of pronounced diffusion, efficiency is lost together with analysis time. Right from the minimum efficiency is lost, but analysis is speeded up. If separation is sufficient, this higher velocity range might be chosen. If separation is critical, the singular velocity with highest efficiency should be adjusted.

1.3 Chromatographic Resolution

The characteristic number, which describes the extent of separation of the chromatographic peaks of a pair of solutes, i and j, is the resolution, R_{ji}. The resolution is a measure for the distance of the peak maxima. This distance is not measured in absolute units, but on a scale based on the widths of the peaks.
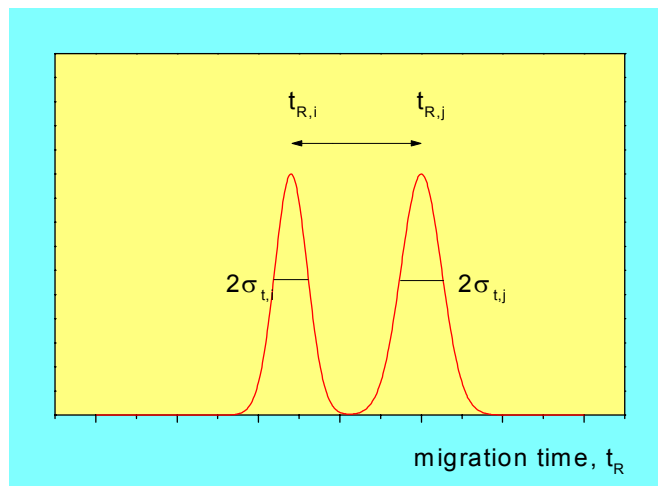
Figure 10 Chromatographic resolution of the two peaks for components i and j.

The generally accepted definition has the form (in the time domain)

$$R_{ji} \equiv \frac{t_{Rj} - t_{Ri}}{2(\sigma_{t,j} + \sigma_{t,i})} \tag{27}$$

It is clear that this definition is not very operational, and therefore this expression is transformed for practical reasons by substitution of the peak width by the plate height and the retention time by the capacity factors. For analytes with similar capacity factors (the analytical case of interest) the following expression for the resolution can be derived, which has a much larger potential for optimisation than the definition given by eq. 27:

$$R_{ji} = \frac{1}{4}\left(\alpha_{ji} - 1\right)\left(\frac{k'}{1 + k'}\right)\sqrt{N} \tag{28}$$

α_{ji} is the selectivity coefficient defined as

$$\alpha_{ji} = \frac{k'_j}{k'_i} \tag{29}$$

It can be seen that the resolution consists of three terms:

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⇨   the selectivity term (α_{ji} -1),

⇨   the retardation term $k'/(1+k')$, and

⇨   the efficiency term, $\sqrt{N}$.

What is the significance of these three terms for the adjustment of the resolution?

(i)   The retardation term - having less importance in HPLC or in GC with packed bed columns – can be of interest in capillary GC, especially when highly volatile solutes are to be separated, which may possess low k` values. The dependence of this term on the retention (or capacity) factor is shown in Figure 11.
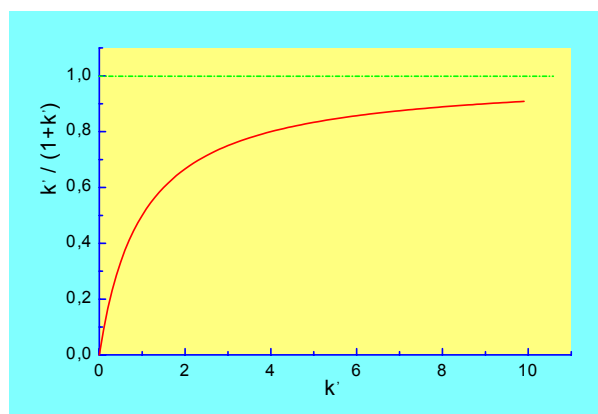

Figure 11 Dependence of the retardation term in the resolution equation on the capacity factor, k`.

It can be seen that the value of this term is small at low $k`$, and influences therefore the resolution unfavourably in this range. The term approximates unit value at large $k`$. It follows that it is of advantage to establish not too low $k`$ values. In GC this can be done e.g. by decreasing the working temperature of the column, or by the appropriate selection of the kind of the stationary phase or its film thickness. In LC it is adjusted by the selection of stationary and mobile phase as well.

(ii)   The third term of the resolution equation contains the plate number. If the resolution is not sufficiently large, enlargement of the column length increases N. As N, and therefore L, are found under the square root in the resolution equation only, an increase of the column length by a factor of 2 leads only to an increase of the resolution by the factor of 1.4, that is only 40 %. This improvement in resolution must be paid, however, by the double increase of the analysis time.

(iii)   The selectivity term is that with the highest potential for optimisation. This holds especially for those pairs of analytes, which are crucial to separate, because they exhibit very similar capacity factors, and elute close to each other. In this case the selectivity coefficient α_{ji} is very close to unit. However, especially in this case minute changes of the selectivity coefficients (e.g. from 1.05 to 1.10, less than 5 %) can result in drastic changes of the selectivity term (which is α -1); in the given example the term changes from 0.05 to 0.1, this is 100 %). In the same way the resolution is increased here strongly by a factor of 2.

1.4   Additivity of variances

It was seen that the total plate height generated due to the chromatographic process is the sum of the increments of the plate heights stemming from the individual contributions: longitudinal diffusion, convective mixing, slow kinetics of mass exchange in the mobile and in the stationary phase, respectively. This summation can be done because the chromatographic system behaves linearly, with other words, because the processes responsible for the individual contributions are acting independently from each other. This finding can be expressed also as the result of system theory, by the additivity of variances. The variance is the second moment of the distribution function (the chromatographic peak). In case of normal distribution, it is the square of the standard deviation, σ^2.

The additivity of variance states that the total variance is the sum of the variances of the individual contributions (given that the system behaves linear, see above)

$$\sigma_{tot}^2 = \sum \sigma_{ind}^2 \qquad (30)$$

As the plate height is proportional to the variance (see eq. 19) the additivity of the variances allows deriving eq. 21 for the incremental addition of the individual contributions to band broadening, which leads to the equivalent expression

$$H_{tot} = \sum H_{ind} \qquad (31)$$

For peak broadening in chromatography it reads

$$\sigma_{chrom}^2 = \sigma_{diff}^2 + \sigma_{con}^2 + \sigma_{ex,m}^2 + \sigma_{ex,s}^2 \qquad (32)$$

Furthermore, the additivity of variances also enables expressing plate heights for effects in addition to chromatographic ones being responsible for peak dispersion, e.g. so-called extra-column effects. Then the total variance is the sum:

$$\sigma_{tot}^2 = \sigma_{chrom}^2 + \sigma_{ex}^2 \qquad (33)$$

These effects may stem from finite injection and detection volumes, from dead volumes of tubings, etc. Although not Gaussian shaped in general, in many cases the variances from defined functions can be simply expressed analytically.

Extra-column Effects

Sources	Shapes
finite injection zone, detection volumes, dead volumes (tubings, etc.)	Arbitrary, Gaussian, rectangular, exponential, triangular

Function	Width	Variance	Example
Gaussian	σ	σ^2	Chromatographic column
Rectangular	τ	$\tau^2/12$	Injector, detector
Exponential	τ	τ^2	Mixing chamber (injector, tubings)

1.5 Detectors: General criteria of performance

We include here a discussion on general characteristics of detectors, although this topic is not limited to chromatographic detectors. Properties of detectors are even not part of the theory of

chromatography in general. However, detector characteristics are an important aspect in chromatographic practice.

Principally one can distinguish two detector types:

(i)   mass flow dependent and

(ii)   concentration dependent detectors. For mass flow dependent detectors the signal S_i is proportional to the change of the mass m with time t

$$S_i^m = a_i^m \frac{dm_i}{dt} = a_i^m m'$$ (34)

Concentration dependent detectors exhibit proportionality of the signal to concentration c

$$S_i^c = a_i^c c_i = a_i^c \frac{dn_i}{dV}$$ (35)

1.5.1 Sensitivity

The factor of proportionality, a_i, in eqs. 34 and 35 is named the sensitivity of the detector

$$a_i^m = \frac{S_i^m}{m'}$$ (36)

or

$$a_i^c = \frac{S_i^c}{c_i}$$ (37)

Mass flow dependent detectors give peak areas, which are independent of dilution of the sample with mobile phase, in contrast to concentration dependent ones.

It should be noted that the detector sensitivity in a strict sense describes the change of the signal generated by a certain change of concentration or mass flow. This is clearly indicated in Figure 12: the sensitivity is the slope of the calibration curve. In the linear range the sensitivity is constant, and independent of concentration. Outside the linear range it is still defined: it is the tangent on the S vs. c or m' curve (dashed line). Note that the sensitivity is not to be confused with the ability to determine small amounts of analyte.
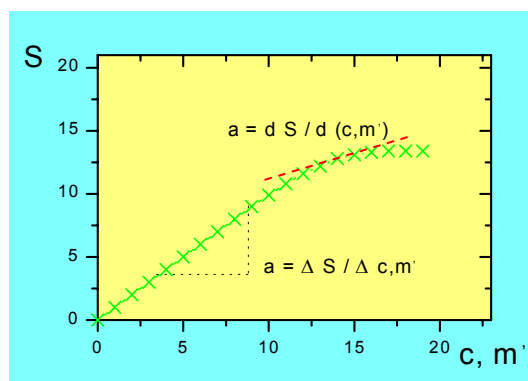
Figure 12 Calibration curve showing the dependence of the signal, S_i, on the concentration, c, of the analyte, or its mass flow, m`, respectively.

1.5.2   Linearity

The linear range is that concentration range where the sensitivity is constant and independent of concentration. In this range the calibration function (S vs. c or m`) is linear. Outside the linear range the sensitivity is still defined: it is the tangent on the $S$ vs. $c$ or $m$` curve. A large linear range is desirable. However, the availability of advanced calibration software in the last years reduced the importance of this parameter. It should be noted that at high concentration deviation from linearity (either convex or concave) is normal. Note further that deviation from linearity does not necessarily hinder quantitative analysis.

1.5.3   Noise

The detector signal is always accompanied by a statistical fluctuation of the measuring values, the noise. This fluctuation has electronic, thermal or e.g. chemical reasons. Due to its statistical nature the amplitude of the detection signal is Gaussian distributed. The width of this distribution is typically characterised by the standard deviation, σ_o (suffix 0 indicates the blank signal).
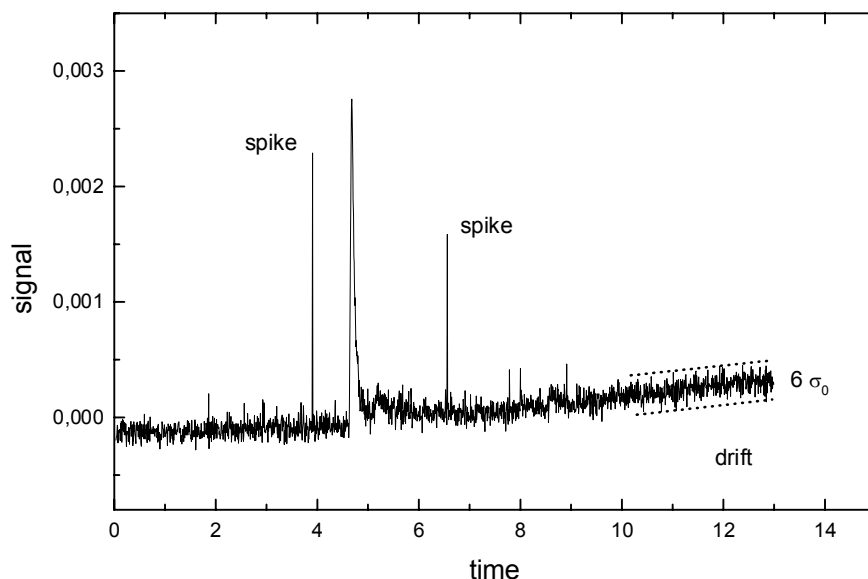
Figure 13 Chromatogram with noise, spikes and baseline drift.

1.5.4 Instrumental limit of detection

The limit of detection, z_i, is that lowest concentration, or smallest mass flow, which can be distinguished from the noise by a certain predefined probability.

$$z_i = \frac{b\sigma_0}{a_i} \tag{38}$$

b is that manifold of the standard deviation of the noise, which is preselected according to statistical reasons. The larger the value for b is taken, the larger is the statistical certainty that a particular signal is not stemming from the noise, with other words, to originate from the sample. If the value for b is e.g. 3, this statistical certainty is 99.7 % (for 3 σ_o). This means that in 1000 cases the signal is stemming 997 times from the sample, in 3 cases it is mimicked from the noise. It comes out that it is the relation of signal to noise (the signal to noise ratio) rather than the sensitivity that determines the ability of a detector to detect low amounts of analyte.

1.5.5 Specifity

The specifity, E , of a detector describes its ability to respond to different substances (at the same concentration or mass flow) with different signal values. The specifity is expressed for two substances i and j by

$$E_{ij} = \frac{a_i}{a_j} \tag{39}$$

Special relevance has the case where E is either 1 or ∞, which, however, does not occur in practice in general.

For $E=1$ all components are detected with equal sensitivity. The detector is universal, and quantitative analysis can be carried out most simply, because all quantities can be determined with one and the same calibration function.

For trace analysis the case is often most desirable that the detector response for matrix components is small, because then the specifity is large. In the extreme case E is infinitely large, i.e. only one single analyte is detected.

1.5.6 Time response

The time behaviour of the detector (including the units connected for data collection and processing) plays a more important role in capillary GC than e.g. in HPLC or in GC with packed columns. The peaks in the former method can be very narrow due to the high plate number reachable here, and the time the peaks are passing the detector might be very short thus.

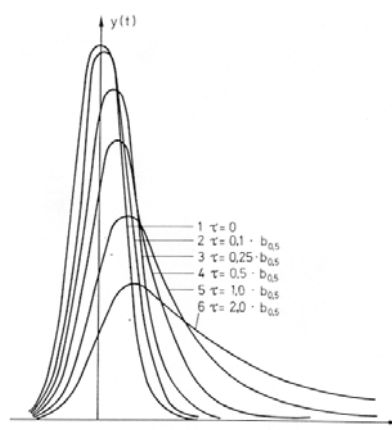The time properties influence the peak shape, but not the peak area (Figure 14).

Figure 14 Peak shape obtained with different time constants, τ. $b_{0,5}$ = width at halve height.

The time property can be described by the time constant, τ. Note that the property of the entire measuring unit is decisive. The time constant is a measure for the distortion of the profile of the measured values (the output signal of the detector) resulting from the given concentration profiles (the input into the detector). Experimentally the time constant can be

determined by the aid of certain measuring functions, e.g. by a rectangular concentration step of a sample added to the mobile phase.
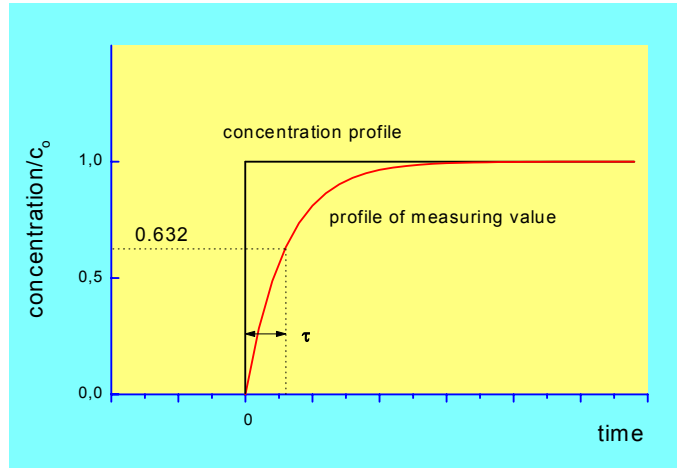

Figure 15 Time response of detector; τ, time constant

A stepwise change of the concentration of a reference compound from zero to c_o occurs in the mobile phase at time $t = 0$. The concentration, c_D , measured with the detector as function of time can be described for the case of a first order response by the function

$$c_D = c_0 \left(1 - e^{-\frac{t}{\tau}} \right)$$
(40)

The time constant τ is the time the detector signal follows the concentration step to the value $\left(1 - e^{-1}\right)c_0 = 0.632\,c_0$.

1.6   Further readings

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