

Chapter 2

Theory of Gas Chromatography

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Abstract This chapter deals with the basic theory of GC. Instead of presenting an in-depth theoretical background, we kept the theory, in particular the equations, to a minimum, restricting it to the most fundamental aspects needed to understand how gas chromatography works. Whenever possible, the practical consequences for the application in the laboratory are discussed. Retention parameters, separation factor, resolution, peak capacity, band broadening including plate theory, as well as van Deemter and Golay equations are discussed. Furthermore, aspects of selecting the optimum gas flow rate are reviewed.

2.1 Introduction

In chromatography, we face a number of fairly complex interactions and processes that cannot be completely predicted or calculated a priori. However, using a number of assumptions, we can simplify these complex processes and reduce them to general principles that can be described sufficiently. Different theories and models have evolved that are applicable and valid under the given assumptions. These models are not only useful to explain the chromatographic process from a theoretical point of view, but they also offer valuable input for the practical application of gas chromatography. In this chapter, we do not intend to give an in-depth introduction into chromatographic theory. We rather aim to present a thorough synopsis of the chromatographic basics that are needed to understand the chromatographic process and that provide helpful input for the GC user in praxis.

We have to consider two basic phenomena for the chromatographic separation of a mixture: the separation of the substances and the broadening of the substance bands. (The substance or chromatographic band is the mobile phase zone containing the substance and corresponds to its peak in the chromatogram.) The separation is caused by distinct migration rates of the components due to differently strong interactions with the stationary phase. This separation is superimposed with mixing processes (dispersion) during the transport through the column, which cause a broadening of the substance bands and counteract the separation since broad bands/peaks impede the resolution of closely eluting peaks. Consequently, we aim to sufficiently maximize the differences in migration rates and minimize the dispersion of the components by choosing appropriate column dimensions and operating parameters.

The migration rate of a compound is the sum of the transport rate through the column and the retention in the stationary phase. The time spent in the mobile phase is the same for all sample components, but the retention is compound specific. It is

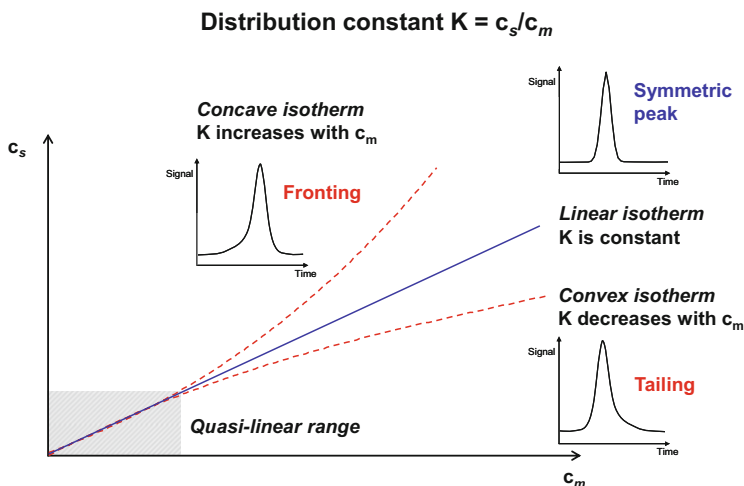


Fig. 2.1 Correlation between the shape of the distribution isotherm and peak form. Adapted and modified from [1]

based on the distribution of an analyte between stationary and mobile phase and is expressed by the distribution constant K . Since the mobile phase is a gas in GC the distribution of a component takes place either between a highly viscous and high boiling liquid and the gas phase, which is called gas-liquid chromatography (GLC), or between the surface of a solid and the gas phase called gas-solid chromatography (GSC).

The distribution constant is defined as

$$K = c_s/c_m \quad (2.1)$$

c_s concentration of a component in the stationary phase

c_m concentration of a component in the mobile phase

A separation is only successful if the distribution constants of the sample components are different. The bigger K the longer stays the component in the stationary phase and the slower is the overall migration rate through the column. The distribution constant can be graphically described with a distribution isotherm with the concentration of the solute in the mobile and stationary phase as x - and y -axis, respectively. The distribution constant is either independent of the concentration of the component (linear isotherm) or changes with the concentration (nonlinear isotherm). In the latter case, the effective migration rate depends on the concentration, which results in unsymmetrical solute bands. Figure 2.1

demonstrates how the concentration profile or peak form of the moving solute band is influenced by the shape of the distribution isotherm.

A linear isotherm delivers a symmetric solute band (peaks) and the peak maximum is independent of the solute amount. A nonlinear isotherm results in unsymmetrical solute bands and the location of peak maximum depends on the solute amount. A nonlinear isotherm can either be formed convex or concave. In case of a concave isotherm, K increases with increasing concentrations resulting in a shallow frontal edge and a sharp rear edge of the peak. This is called fronting. As a consequence, the peak maximum moves to higher retention times (see Chap. 7, Fig. 7.2). In the opposite case, the convex isotherm, K , decreases with increasing concentrations resulting in a sharp frontal edge and a shallow rear edge of the peak. This is called tailing. The peak maximum moves to lower retention times. In practice, linear distribution isotherms are only found if the solute and stationary phase are structurally similar. However, as Fig. 2.1 shows, even for nonlinear distribution isotherms, a quasi-linear range exists at low concentration, which delivers symmetric peaks with retention times that are independent of the solute amount. **One should keep in mind to work at low concentrations in the quasi-linear range if retention values are used for identification (see Chap. 7).**

Depending on the shape of the distribution isotherm, we distinguish between linear and nonlinear chromatography for the description of chromatographic processes. We further divide into ideal and nonideal chromatography. Ideal chromatography implies a reversible exchange between the two phases with the equilibrium being established rapidly due to a fast mass transfer. Diffusion processes that result in band broadening are assumed to be small and are ignored. In ideal chromatography the concentration profiles of the separated solute should have a rectangle profile. The Gaussian profile obtained in practice demonstrates that these assumptions are not valid. In case of nonideal chromatography these assumption are not made. With these two types of classification the following four models are obtained to mathematically describe the process of chromatographic separation:

- Linear, ideal chromatography
- Linear, nonideal chromatography
- Nonlinear, ideal chromatography
- Nonlinear, nonideal chromatography.

In GC, the mostly used partition chromatography can be classified as linear nonideal chromatography. In that case, almost symmetric peaks are obtained and band broadening is explained by the kinetic theory according to van Deemter [2].

2.2 Retention Parameters

The nomenclature and symbols used in the literature to describe retention parameters are rather inconsistent, which can be confusing especially while reading older papers. In 1993 a completely revised “Nomenclature for Chromatography”

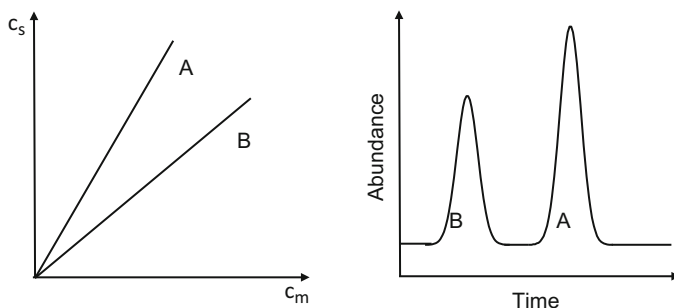


Fig. 2.2 Correlation between distribution constant and peak position

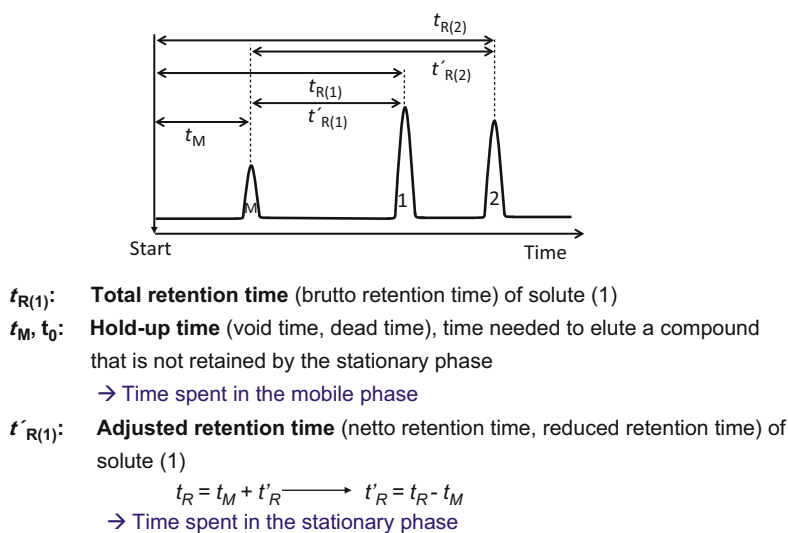


Fig. 2.3 Elution chromatogram with start (sample injection), baseline, hold-up time (t_M), retention time (t_R), and adjusted retention time (t'_R)

was published by the IUPAC [3] and we will mostly follow these recommendations. A summary of the IUPAC nomenclature together with additional and outdated terms is given in the appendix.

As already mentioned, the chromatographic separation of mixture is based on the different distribution of the components between the stationary phase and the mobile phase. A higher concentration in the stationary phase results in a longer retention of the respective solute in the stationary phase (Fig. 2.2). A separation requires different values of the distribution constants of the solutes in the mixture.

Let's first just consider one solute. The time spent in the chromatographic column is called retention time t_R based on the Latin word *retenare* (*retain*). Figure 2.3 shows a schematic elution chromatogram with the detector signal (y axis) as function of time (x axis).

The detector signal is proportional to the concentration or mass of the solute in the eluate leaving the column. With older recorders the signal was measured in mV while modern computer-based systems deliver counts or arbitrary units of abundance. If no solute is leaving the column an ideally straight line the so-called baseline is recorded, which is characterized by slight fluctuations called the baseline noise (see also Chap. 6). If a solute is leaving the column, the baseline rises up to a maximum and drops then back down again. This ideally symmetric shape is called a chromatographic peak. (Please note that signals in mass spectrometry likewise are called peaks, but they are representation of abundance in mass to charge ratio.) The chromatogram delivers the following basic terms:

The time that passes between sample injection (starting point) and detection of the peak maximum is called retention time t_R and consists of two parts:

- The time spent in the stationary phase called adjusted retention time t'_R or outdated net-retention time
- The time spent in the mobile phase called hold-up time t_M , dead time, or void time

$$t_R = t_M + t'_R \quad (2.2)$$

The hold-up time t_M is the time needed to transport the solute through the column, which is the same for all solutes in a mixture. The hold-up time can be determined by injecting a compound, an inert or marker substance, that is not retained by the stationary phase, but that can be detected with the given detection system, e.g.:

Detector	$t_M = t_R$ of an inert substance
FID	Methane, propane, butane
WLD	Air, methane, butane
ECD	Dichloromethane, dichlorodifluoromethane
NPD	Acetonitrile
PID	Ethylene, acetylene
MS	Methane, butane, argon

In reality, the compounds listed above are not ideal inert substances. Depending on the chromatographic column, the conditions must be chosen in a way that the retention by the stationary phase is negligible, e.g., by using higher oven temperatures. The hold-up time can also be determined based on the retention time of three consecutive n -alkanes or other members of a homologous series:

$$t_M = \frac{t_{R(z)} \times t_{R(z+2)} - t_{R(z+1)}^2}{t_{R(z)} \times t_{R(z+2)} - 2t_{R(z+1)}} \quad (2.3)$$

z	carbon number of n -alkanes
$t_{R(z)}$	retention time of n -alkane with carbon number z
$t_{R(z+1)}$	retention time of n -alkane with carbon number $z+1$
$t_{R(z+2)}$	retention time of n -alkane with carbon number $z+2$

Even better, linear regression is performed:

$$\log t'_{R(z)} = \log(t_R - t_M) = a + bz \quad (2.4)$$

Furthermore, t_M can be calculated based on the column dimensions and carrier gas pressure:

$$t_M = \frac{32L^2\eta(T)}{3r^2} \cdot \frac{p_i^3 - p_o^3}{(p_i^2 - p_o^2)^2} \quad (2.5)$$

L	column length
r	column inner radius
$\eta(T)$	viscosity of the carrier gas at column temperature T
p_i	column inlet pressure (see also note for eq. 2.56)
p_o	column outlet pressure (atmospheric pressure)

Software tools are available from major instrument manufacturers, such as the flow calculator from Agilent Technologies [4], which can be used to calculate t_M .

The adjusted retention time (t'_R) depends on the distribution constant of the solutes and therefore on their interactions with the stationary phase. Furthermore, retention times are influenced by the column dimensions and the operation conditions (column head pressure, gas flow, temperature). The reproducibility of these parameters was quite limited in the early days of gas chromatography, but has improved tremendously with the modern instruments in use these days.

Multiplying the retention time with the gas flow F_c of the mobile phase results in the respective volumes: retention volume, adjusted retention volume, and hold-up volume:

$$V_R = t_R \times F_c \quad (2.6)$$

$$V'_R = t'_R \times F_c \quad (2.7)$$

$$V_M = t_M \times F_c \quad (2.8)$$

$$V_R = V_M + V'_R \quad (2.9)$$

where F_c is the carrier gas flow at the column outlet at column temperature.

2.2.1 Retention Factor

A more reproducible way to characterize retention is the use of relative retention values instead of absolute values. The retention factor k , also known as capacity

factor k' , relates the time a solute spent in the stationary phase to the time spent in the mobile phase:

$$k = t'_R/t_M \quad (2.10)$$

The retention factor is dimensionless and expresses how long a solute is retained in the stationary phase compared to the time needed to transport the solute through the column.

Assuming the distribution constant K is independent of the solute concentration (linear range of the distribution isotherm), k equals the ratio of the mass of the solute i in the stationary ($W_{i(S)}$) and in the mobile phase ($W_{i(M)}$) at equilibrium:

$$k = W_{i(S)}/W_{i(M)} \quad (2.11)$$

The higher the value of k , the higher is the amount of the solute i in the stationary phase, which means the solute i is retained longer in the column. Consequently, k is a measure of retention.

Using Eqs. (2.2) and (2.10) yields

$$t_R = t_M(1 + k) \quad (2.12)$$

With

$$\bar{u} = L/t_M \quad (2.13)$$

we obtain a simple but fundamental equation for the retention time as function of column length, average linear velocity of the mobile phase, and retention factor:

$$t_R = \frac{L}{\bar{u}}(1+k) = \frac{L}{\bar{u}} \left(1 + \frac{K}{\beta} \right) \quad (2.14)$$

L	column length
\bar{u}	average linear velocity of the mobile phase
k	retention factor, $k = K/\beta$
K	distribution constant of a solute between stationary and mobile phase, $K = c_s/c_m$
c_s	concentration of the solute in the stationary phase
c_m	concentration of the solute in the mobile phase
β	phase ratio, $\beta = V_m/V_s$ with volume of the mobile phase (V_m) and volume of the stationary phase (V_s) in the column

The retention time is directly proportional to the column length and indirectly proportional to the average linear velocity of the mobile phase according to this equation. However, we cannot freely choose the average linear velocity of the

mobile phase, as we will discuss in the Sect. 2.4.2, because it has a tremendous influence on band broadening and consequently on the separation efficiency of the column.

2.3 Separation Factor

If two analytes have the same retention time or retention volume on a column, they are not separated and we call this coelution. A separation requires different retention values. The bigger these differences, the better is the separation efficiency or selectivity of the stationary phase for the respective pair of analytes. This selectivity is expressed as separation factor α , also called selectivity or selectivity coefficient. The separation factor α is the ratio of the adjusted retention time of two adjacent peaks:

$$\alpha = t'_{R(2)}/t'_{R(1)} = k_2/k_1 = V'_{R(2)}/V'_{R(1)} = K_2/K_1 \quad (2.15)$$

By definition α is always greater than one, meaning $t'_{R(2)} > t'_{R(1)}$.

The α value required for baseline separation of two neighboring peaks depends on the peak width, which we will discuss in the next section. The ratio $t'_{R(2)}/t'_{R(1)}$ is also called relative retention r if two peaks are examined that are not next to each other. Often, one analyte is used as a reference and the retention of the other analyte is related to this retention standard (see also Chap. 7).

The selectivity of liquid stationary phases is mostly determined by two parameters: the vapor pressure of the solutes at column temperature and their activity coefficients in the stationary phase. The liquid stationary phase can be considered as a high boiling solvent with a negligible vapor pressure and the analytes are dissolved in this solvent. The partial vapor pressure of the solutes is equal to their equilibrium concentration in the gas phase above the solvent. The correlation between the concentration of a solute in solution (liquid stationary phase) and in the gas phase is described by Henry's law:

$$p_i = p_i^\circ \times f_i^\circ \times n_{i(S)} \quad (2.16)$$

p_i	partial vapor pressure of the solute i at column temperature over the solution
p_i°	saturation vapor pressure of the pure solute i at column temperature
f_i°	activity coefficient of the solute i in the solution (stationary phase) at infinite dilution
$n_{i(S)}$	mole fraction of the solute i in the stationary phase (molar concentration)

In the special case of an ideal solution $f^\circ = 1$, Eq. (2.16) turns into Raoult's law. If we assume that adsorption processes at the interfaces are negligible and taking further simplifying assumptions into account (e.g., Henry's law, ideal gas behavior, high dilution), the following equation is derived for the distribution constant K of a solute i :

$$K = c_s/c_m = R \times T/p^\circ \times f^\circ \times V_S = R \times T \times d_S/p^\circ \times f^\circ \times M_S \quad (2.17)$$

- R gas constant
- T absolute column temperature [Kelvin]
- V_S molar volume of the liquid stationary phase
- M_S molecular weight of the liquid stationary phase
- d_S density of the liquid stationary phase

The dependency of $K \sim 1/p^\circ \times f^\circ$ leads us to an important correlation, because the retention factor k ($k = K/\beta$) is then also inversely proportional to the saturation vapor pressure and the activity coefficient of the solute:

$$k \sim 1/p^\circ \times f^\circ \quad (2.18)$$

If we examine the separation of two analytes, we obtain the following equation for the separation factor or the relative retention by combining Eqs. (2.15) and (2.18):

$$\alpha = k_2/k_1 = p_1^\circ f_1^\circ / p_2^\circ f_2^\circ \quad (2.19)$$

The log-transformed version of Eq. (2.19) is the so-called Herington's separation equation [5] that was originally derived for extractive distillation:

$$\log \alpha = \log(k_2/k_1) = \log p_1^\circ / p_2^\circ + \log f_1^\circ / f_2^\circ \quad (2.20)$$

The equation contains two terms: the vapor pressure term, sometimes not quite correctly called boiling point term, and the activity term, which is also called solubility or interaction term. According to Eq. (2.20) two analytes can be separated on liquid stationary phases if they differ in their vapor pressure and/or their activity coefficient in the respective stationary phase. The vapor pressure term depends on the structure of the two analytes and is independent of the chosen stationary phase. However, it is influenced by the column temperature. This term does not contribute to the separation if the two analytes possess the same vapor pressure. A separation is only possible in this case, if the activity coefficients are different. As we will see later in this chapter, the two terms can act concordantly or contrarily. Herington's separation equation also shows that GLC can be used to determine physicochemical parameters such as activity coefficient, vapor pressure, and related parameters.

The activity term expresses the strength of the intermolecular forces between the solvent (stationary phase) and the dissolved solutes. The stronger the force of attraction, the higher is the portion of the solute in the stationary and consequently the retention time. The intermolecular forces depend on the structure of the interacting partners, solvent and solute, such as type and number of functional groups, and spatial alignment (sterical hindrance). Depending on the structure, we distinguish between polar, polarizable, and nonpolar molecules that differ in their ability to form intermolecular forces:

Polar molecules contain heteroatoms and/or functional groups that lead to an unsymmetrical charge distribution and consequently to a permanent electric dipole moment. Examples are ethers, aldehydes, ketones, alcohols, and nitro- and cyano-compounds.

Polarizable molecules are nonpolar molecules that do not possess a permanent electric dipole moment, but in which a dipole moment can be induced by adjacent polar molecules and/or electric fields. This requires polarizable structures in the molecule such as double bonds or aromatic structures.

Nonpolar molecules are molecules without a dipole moment that are not prone to the induction of a dipole moment. Typical examples are the saturated hydrocarbons.

Intermolecular forces are forces of attraction (or repulsion) between valence-saturated, electrical neutral molecules that are in close proximity. The energy of intermolecular forces is much lower (<25 kJ/mol) than the energy of chemical bonds (>400 kJ/mol, intramolecular forces). The interaction energy decreases with increasing distance between the interacting partners; more precisely, it is inversely proportional to the sixth power of the distance. While we are mostly interested in chromatographic retention caused by the intermolecular forces, they are also responsible for the so-called cohesion properties such as melting and boiling point, solubility, miscibility, surface tension, and interface phenomena. In gas chromatography the following intermolecular forces (van der Waals force) are important:

2.3.1 Dispersion Forces (London Forces)

These nonpolar forces are weak, nondirected (nonspecific) forces between all atoms and molecules. They are always present both for nonpolar and polar molecules. They can be explained with the fluctuating dipole model. Dispersion forces increase with the molecular mass of the molecules, which results in a higher boiling point.

2.3.2 *Induction Forces (Dipole-Induced Dipole, Debye Forces)*

Induction forces are directed forces between polar molecules (molecules with dipole) and polarizable molecules.

2.3.3 *Dipole–Dipole Forces (Keesom Forces)*

Dipole–dipole forces are directed forces between polar molecules (molecules with a permanent dipole).

2.3.4 *Hydrogen Bonding*

The hydrogen bond is the strongest electrostatic dipole–dipole interaction:



where XH is the proton donator, e.g., $-OH$, $-NH$, and IY the proton acceptor (atoms with free electron pairs, electron donators).

The strength of the interaction forces increases from dispersion, over induction to dipole forces. Induction and dipole forces are often called polar interactions. The strong dipole and hydrogen bond forces are for example responsible for the high boiling point of small polar molecules such as ethanol or acetonitrile.

2.3.5 *Electron–Donor–Acceptor Interactions*

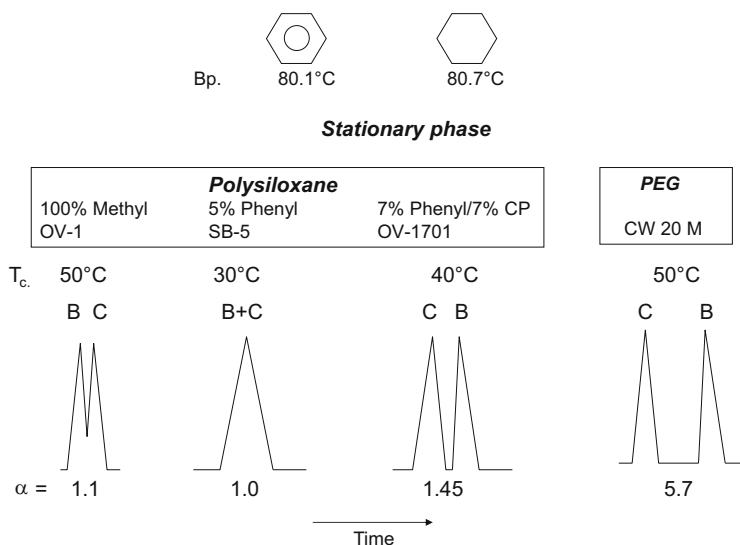
Interaction between molecules with electron donor and acceptor properties due to electron transfer from the highest occupied to lowest unoccupied orbital, e.g., nitro- or cyano-compounds as electron acceptors and aromatics as electron donors.

In practice, the interaction energy, meaning the strength of the attraction force, is determined by the sum of interactions. Table 2.1 demonstrates that stationary phases with different functional groups are capable to undergo diverse interactions resulting in variable retention properties.

Figure 2.4 demonstrates how the polarity of the stationary phase influences the separation (characterized by the separation factor α) and the elution order using the separation of benzene (B) and cyclohexane (C) on different stationary phases as example. The vapor pressure term of Herington's separation equation delivers only a minimal contribution to the separation because the boiling points of the two cyclic

Table 2.1 Functional groups and potential interactions. Reproduced with permission from [6]

Stationary phase:			
Functional groups	Potential interactions		
	Dispersion/induction	Dipole	H-bond
Methyl	Strong	None	None
Phenyl	Very strong	None	Weak
Cyanopropyl	Strong	Very strong	Medium
Trifluoropropyl	Strong	Medium	Weak
Polyethylene glycol	Strong	Strong	Moderate

Separation of benzene (B) and cyclohexane (C)**Fig. 2.4** Column polarity and elution order; Bp. boiling point, CP cyanopropyl, PEG polyethyleneglycol

hydrocarbons are almost identical. Both hydrocarbons are nonpolar, but an unsymmetrical charge distribution can be induced in benzene due to the easily shiftable π -electrons. Therefore, benzene is capable to form induction interactions with polar stationary phases. On the nonpolar phase OV-1 (100 % dimethylpolysiloxane, see Chap. 3) only an incomplete separation is achieved. A better separation would require a higher plate number. The elution of benzene before cyclohexane takes place according to their boiling points. Due to the delocalized π -electrons in the phenyl groups, the often used 5 % phenyl methylpolysiloxane phase (SB-5) can undergo induction interactions and is therefore slightly polar. Interestingly, the two hydrocarbons are not separated by this phase. Apparently, the vapor pressure and the solubility term compensate each other, that is, both terms are equal but with opposite signs. In contrast, the two other phases – methylpolysiloxane with 7 %

Table 2.2 Column polarity and elution order

Nonpolar stationary phase: (nonpol. SP)		– Squalane – 100 % dimethylpolysiloxane (OV-1)		
Polar stationary phase: (pol. SP)		– Methylpolysiloxane with 7 % phenyl and 7 % cyanopropyl (OV 1701) – Polyethylene glycol (PEG, Wax)		
Compound	Formula	Bp. (°C)	Nonpolar SP	Polar SP
Benzene	C ₆ H ₆	80.1	1. Peak	2
Cyclohexane	C ₆ H ₁₂	80.7	2. Peak ^a	1
Ethanol	C ₂ H ₅ OH	78.4	1	3
2,2-Dimethylpentane	C ₇ H ₁₆	79.0	2	1
Benzene	C ₆ H ₆	80.1	3	2
Chloroform	CHCl ₃	61.2	1	2
Carbon tetrachloride	CCl ₄	76.7	2	1
Methanol	CH ₃ OH	64.7	1	3
Methyl acetate	CH ₃ COOCH ₃	57.0	3	2
Diethyl ether	C ₂ H ₅ OC ₂ H ₅	34.6	2	1
1-Propanol	C ₃ H ₈ O	97.0	1	4
2-Butanone (MEK)	C ₄ H ₈ O	79.6	2	3
Tetrahydrofuran (THF)	C ₄ H ₈ O	66.0	3	2
<i>n</i> -Heptane	C ₇ H ₁₆	98.4	4	1

^aNonpolar SP requires high plate number for separation

phenyl and 7 % cyanopropyl and polyethylene glycol (PEG) – are much more polar and retain benzene stronger, which is expressed in high α values. Please note, the elution order of benzene and cyclohexane on polar stationary phases does not follow the boiling point order any longer. By modifying the column polarity, we can systematically change the elution order. This can be helpful in trace analysis if minor target analytes are overlapped by large peaks. Thus, benzene (Bp. 80.1 °C) has an extremely high retention on the very polar phase tris-cyanoethoxy-propane (TCEP) resulting in an elution even **after** *n*-dodecane (Bp. 216 °C). TCEP contains 3 cyano groups and possesses strong electron acceptor properties, but the maximum temperature of this stationary phase is only 150 °C.

Let us now examine the separation of chloroform CHCl₃ (Bp. 61.2 °C) and carbon tetrachloride CCl₄ (Bp. 76.7 °C). On a nonpolar stationary phases, the solutes leave the column according to their boiling points as expected. However, the elution order is reversed at polar stationary phases: carbon tetrachloride, 15 °C higher boiling solvent leaves the column first. Again, this demonstrates the opposite effects of vapor pressure and solubility term on polar columns. In this case, the solubility term delivers a higher contribution. This is caused by the strong electro-negativity of the three chloro atoms in chloroform resulting in an unsymmetrical charge distribution in the molecule. Hence, chloroform is a potent partner for strong interactions with polar stationary phases.

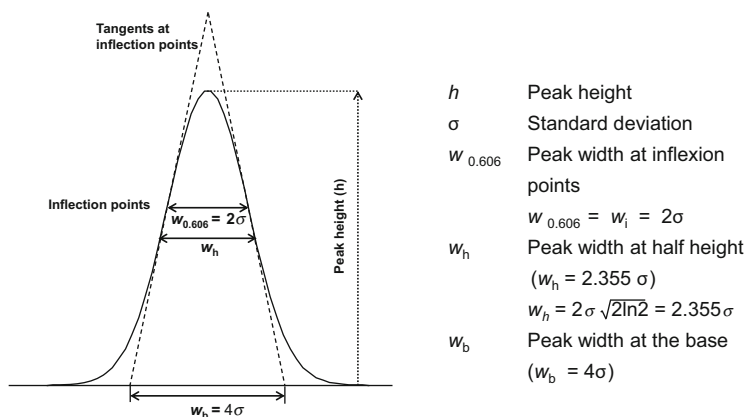
Further examples for the influence of column polarity on the elution order and/or the interplay of vapor pressure and solubility term are given in Table 2.2.

The interpretation of different elution orders can be supported by the so-called similarity rule: *similia similibus solvuntur* (Latin: Similar will dissolve similar). Accordingly, compounds are the better soluble or miscible the more similar their chemical structure. Nonpolar solutes are better dissolved in nonpolar stationary phases and polar solutes in polar stationary phases, respectively. Good solubility corresponds to high retention values and symmetrical peaks.

2.4 Band Broadening

As already mentioned, the width of a chromatographic peak is the result of various mixing processes during the transport of the solute through the column. Consequently, not all molecules of a solute reach the detector at same time, which would result in a narrow rectangular profile, but dispersion around the peak maximum is obtained. This time-dependent concentration profile has a characteristic bell shape and can be described in close approximation by a Gaussian curve (Fig. 2.5).

Assuming a Gaussian profile, the peak width can be determined at different heights. At the inflection points (60.6 % of the peak height), the peak width equals two standard deviations (σ) and the peak width at the base w_b equals 4σ (distance between the intersection of the tangents from the inflection points with the baseline). The peak width at half height is $w_h = 2.355\sigma$. This often used parameter dates back to the time when peak areas were not determined electronically, but peak widths were measured by hand using a ruler and plotting paper. Peak widths are given in units of time or volume.



Peak widths are given in the dimension of retention (time or volume units).

Fig. 2.5 Gaussian profile of a peak. Adapted from [3]

2.4.1 Plate Theory

The plate theory was first introduced to partition chromatography by James and Martin in 1952 [7]. This concept is borrowed from the performance description of distillation columns. It divides the continuous separation process in a number of discrete individual steps. Thus, the column consists of many consecutive segments, called theoretical plates, and for each plate an equilibrium between the solute in the stationary and mobile phase is assumed. The smaller a segment or the height of theoretical plate, the more plates are available per column meter. Consequently, more distribution steps can be performed resulting in less relative band broadening in relation to the column length. The number of theoretical plates N and the height of a plate H are derived from the chromatogram using the retention time of a test solute and a measure for the peak width:

$$N = (t_R/\sigma)^2 \quad (2.21)$$

$$N = 16(t_R/w_b)^2 \quad (2.22)$$

where σ is the standard deviation, w_b is the peak width at base and

$$N = 5.545(t_R/w_h)^2 \quad (2.23)$$

where w_h is the peak width at half height.

The conversion between the different peak heights assumes a Gaussian peak (see Fig. 2.5). The plate height (H) is obtained by dividing the column length (L) by the plate number (N):

$$H = L/N \quad (2.24)$$

The plate height is also often called the height equivalent to one theoretical plate (HETP). The plate height is an important criterion to judge the efficiency of a column and can be used to compare columns. High-quality columns are characterized by a high N and a low H . However, both values depend on the column temperature, the average carrier gas velocity, and the solute, which should always be specified. Keep in mind, N and H are determined under isothermal conditions (validity of the plate theory).

A disadvantage of the often used plate model are the simplifications made to develop the model. Most of all, chromatography is a dynamic process and a complete equilibrium is not reached, but we work under nonequilibrium conditions. Consequently, the plate number is in reality not equal to the number of equilibrium steps reached in the column. The impact of H is rather obtained by the peak width (standard deviation σ) in relation to the length of solute movement L or the retention time [8]:

$$H = \sigma^2/L \quad H = \sigma^2/t_R \quad \sigma = \sqrt{H \times t_R} \quad (2.25)$$

In that case, the height of a theoretical plate expresses the extent of peak broadening in a column for a peak with the retention time t_R . It also shows that the peak width (σ) is proportional to the square root of the retention time [8].

The calculation of N and H uses the retention time that contains also the hold-up time which is not contributing to the separation of the solutes. Therefore, the adjusted retention time is sometimes used to calculate effective plate number and the effective plate height:

$$N_{\text{eff}} = (t'_R/\sigma)^2 \quad (2.26)$$

$$H_{\text{eff}} = L/N_{\text{eff}} \quad (2.27)$$

While the plate theory delivers a value to judge the efficiency of a column, it does not explain peak broadening. This was first achieved with the rate theory by van Deemter [2].

2.4.2 Rate Theory According to van Deemter

The rate theory was introduced by van Deemter [2]. It views the separation process in a packed GLC column under isothermal conditions as a dynamic process of independent mass transfer and diffusion processes that cause band broadening.

Molecular diffusion (derived from the Latin word *diffundere* = spread, disperse) describes the random movement of molecules in fluids, such as gases and liquids. If the movement is driven by concentration differences it is called transport diffusion or ordinary diffusion. In that case, more molecules move from the regions of high concentration to regions of low concentration until the concentration difference is balanced. The rate of this movement is directly proportional to the concentration gradient and in binary systems is expressed as diffusion coefficient D (m^2/s). The diffusion coefficient in gases ranges between 10^{-4} and 10^{-5} m^2/s , while it is 4–5 orders of magnitude lower in liquids (10^{-9} m^2/s). The so-called van Deemter equation describes the relation of the height of a theoretical plate H and the average linear velocity of the mobile phase. In condensed form is expressed as follows:

$$H = A + B/\bar{u} + C\bar{u} \quad (2.28)$$

- H height of a theoretical plate
- \bar{u} average linear velocity of the mobile phase
- A eddy diffusion term
- B longitudinal diffusion term
- C mass transfer term

The average linear velocity of the mobile phase $\bar{u} = L/t_M$ is not identical with the flow rate at the column head or column outlet due to the compressibility of gases and the resistance of the column packing (see Sect. 2.8). The A , B , and C terms represent the contribution of the above-discussed processes to band broadening and should be kept as low as possible.

The **A term** refers to band broadening caused by dispersion (multi-pathway) effects, the so-called Eddy diffusion:

$$A = 2\lambda d_p \quad (2.29)$$

λ correction factor for the irregularity of the column packing
 d_p average particle diameter

The **B term** represents band broadening by longitudinal diffusion, the molecular diffusion both in and against the flow direction:

$$B = 2\gamma D_G \quad (2.30)$$

γ labyrinth factor of the pore channels ($0 < \gamma < 1$)
 D_G diffusion coefficient of the analyte in the gas phase

The **C term** refers to band broadening caused by solute delay due to the mass transfer:

$$C = 8/\pi^2 \times k/(1+k) \times d_L^2/D_L \quad (2.31)$$

k retention factor
 d_L average film thickness of the stationary phase on the support material
 D_L diffusion coefficient of the analyte in the liquid stationary phase

From the van Deemter equation several conclusions can be drawn that are of high importance for the practical application.

Figure 2.6 shows that the plate height depends on the average linear velocity of the mobile phase. The H/\bar{u} curve is a hyperbola with a minimum for H at $\bar{u}_{\text{opt}} = \sqrt{B/C}$. Differentiating Eq. (2.28) with regard to \bar{u} and setting $dH/d\bar{u} = 0$ yields $\bar{u}_{\text{opt}} = \sqrt{B/C}$ and $H_{\text{min}} = A + 2\sqrt{BC}$. Thus, an optimum average linear velocity of the mobile phase exists for each column at which the highest column efficiency or in other words the narrowest peaks are achieved. The optimum is the result of different dependencies of the A , B , and C terms on the velocity of the mobile phase. The A term is independent of the velocity. The B term decreases with increasing velocities; the impact of longitudinal diffusion is less pronounced at higher flow rates. The C term increases with increasing average linear velocities.

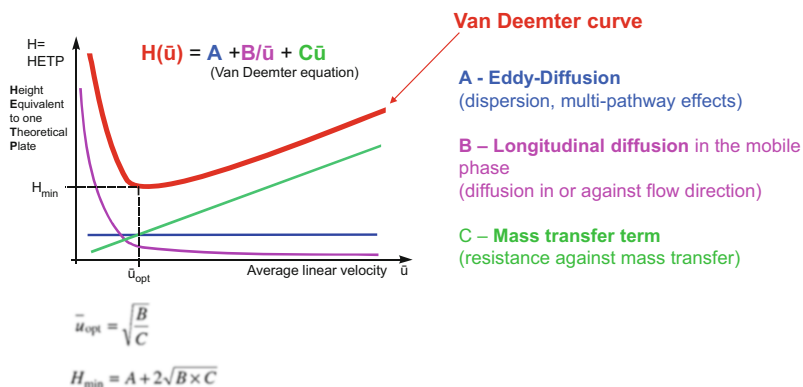


Fig. 2.6 Van Deemter plot showing the contribution of the A, B, and C terms

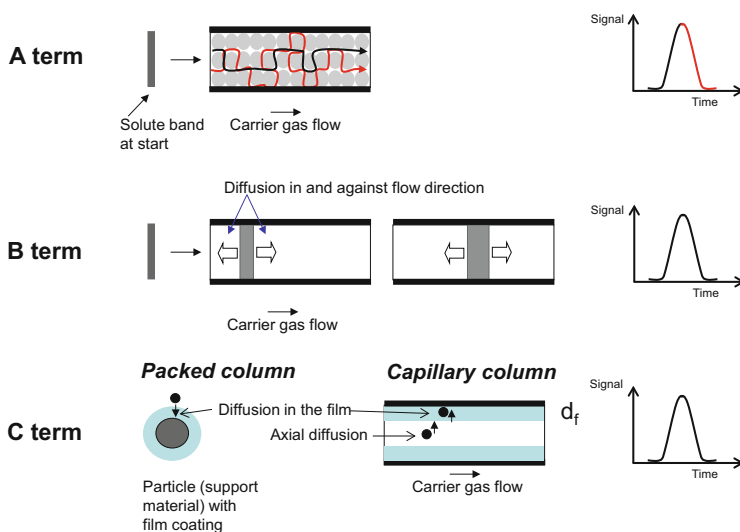


Fig. 2.7 Graphical representation of the A, B, and C terms of the van Deemter equation

As already mentioned the three terms should be as low as possible to achieve small H values (narrow peaks). Let us examine the individual terms a bit more in detail (see also Fig. 2.7).

2.4.2.1 A Term

The transport of the mobile phase through the column packing can occur via different flow channels. In simple terms, molecules belonging to one solute can

take different flow paths around the particles resulting in different pathway lengths and consequently broader peaks. This effect is termed Eddy diffusion. It depends on the particle size and shape as well as the irregularity of the column packing. The higher the diameter and irregularity of the particles the stronger is the dispersion. Consequently, the A term can be minimized using small regular particles and a uniform column packing, but at the cost of a higher backpressure. In addition, at the laminar flow conditions present in chromatography, the flow rate is higher in the middle of the flow channels and lower at the edges.

2.4.2.2 B Term

The B term is directly proportional to the diffusion coefficient D_G of the analytes in the mobile phase. The molecular diffusion overlays the solute transport along the column caused by the pressure drop. The diffusion is caused by concentration differences in the solute band. It is the highest in middle and lower at the beginning and the end resulting in diffusion. The longitudinal component of the diffusion either accelerates the solute transport in longitudinal direction or slows it down. Since diffusion is about 100–1,000-fold faster in gases than in liquids, the B terms has a much higher impact in GC than in LC. As diffusion in gases decreases with increasing molecular mass of the gases, one could conclude that a “heavier” carrier gas is advantageous, but we will see below that this negatively affects the C term.

2.4.2.3 C Term

The C terms refers to the mass transfer between stationary and mobile phase. It is also termed resistance against the mass transport. Chromatography is a dynamic process. A nearly complete partition equilibrium is only reached at very low carrier gas flow rates. Thus, the C term linearly increases with the carrier gas velocity. It takes a finite time to reach equilibrium conditions that include the transport through the mobile phase to the phase interface, the phase transfer (solutes entering the stationary phase), and the transport of the solutes into the liquid stationary phase and back to the phase interface. These transport processes are determined by axial diffusion (perpendicular to the flow direction of the mobile phase). Therefore, the C term is determined by the diffusion coefficients of the solute in mobile and stationary phase and the transport distances, most importantly the thickness of the liquid stationary phase. In contrast to the B term, a fast mass transfer requires high values for the diffusion coefficient. Low molecular weight carrier gases are advantageous for a fast diffusion. As diffusion in liquids is slow, thin films of the liquid stationary phase are beneficial. As initially mentioned, the van Deemter equation was developed for packed columns with liquid stationary phases under isothermal conditions. Later on, it was modified and refined by several researchers (Golay, Huber, Guiochon, Knox, Giddings, and others) taking the specific conditions and requirements of capillary columns, solid stationary phases, and liquid

chromatography into consideration. Furthermore, the compressibility of the carrier gas was taken into account.

The original form of the van Deemter equation [Eq. (2.28) / (2.31)] did not account for band broadening by axial diffusion in the mobile phase (C_M term). Later on, the van Deemter equation was extended to include a C_M term that was introduced by Golay for capillary columns (see below) [9]. The C_M term contains the diffusion coefficient in the gas phase D_G in the denominator:

$$C_M = \omega d_p^2 / D_G \quad (2.32)$$

D_G diffusion coefficient in the gas phase
 d_p average particle diameter
 ω obstruction factor for packed bed

2.4.3 Band Broadening in Capillary Columns: Golay Equation

The equation developed by Golay for capillary columns with liquid stationary phases does not include an A term because these columns do not contain a particulate packing [9]:

$$H = B/\bar{u} + (C_S + C_M)\bar{u} \quad (2.33)$$

A new term (C_M) was introduced to account for band broadening caused by diffusion in the gas phase, which was not included in the original form of the van Deemter equation. Consequently, the Golay equation contains two C terms, C_S and C_M , describing the mass transfer in the stationary and mobile phase. The B , C_S , and C_M terms are defined as follows:

$$B = 2 D_G \quad (2.34)$$

$$C_S = \frac{2k d_f^2}{3(1+k)^2 D_L} \quad (2.35)$$

$$C_M = \frac{(1+6k+11k^2) d_c^2}{96(1+k)^2 D_G} \quad (2.36)$$

k retention factor
 d_f film thickness of the stationary phase
 D_L diffusion coefficient of the analyte in the liquid stationary phase
 d_c column diameter
 D_G diffusion coefficient of the analyte in the gas phase

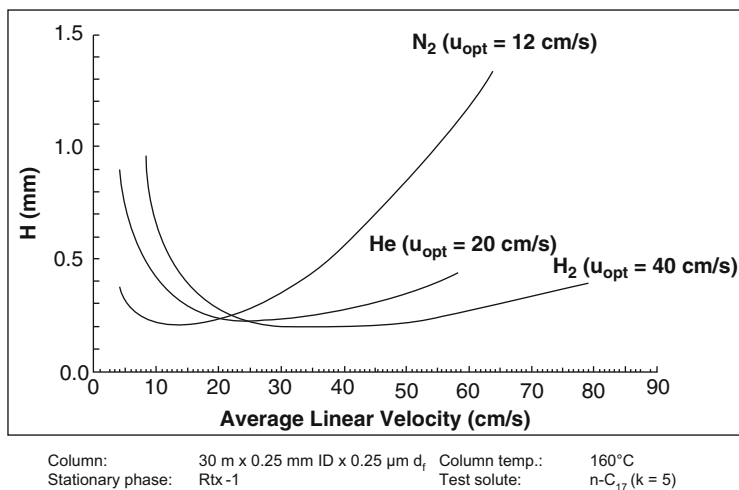


Fig. 2.8 H/\bar{u} plots for different carrier gases. Modified, taken with permission from [10]

As discussed above for packed columns, the B term can be reduced by using a higher molecular weight carrier gas, such as nitrogen or argon. But this is contradictory to the desired fast mass transfer requiring a fast diffusion orthogonal to the flow direction of the mobile phase. The contribution of the B term to band broadening can be reduced by increasing the linear velocity of the carrier gas, but concomitantly the C terms increase.

The most important determinants of the C_S term are film thickness and diffusion coefficients of the analytes in the mobile phase. The C_S term can be reduced by thin films and stationary phases with a low viscosity. The C_M term is determined by the inner diameter of the capillary and the diffusion coefficient of the analytes in the mobile phase. Since the diffusion rate decreases in gases with a higher molecular mass, different H/\bar{u} plots are obtained for a given column if different carrier gases are used. This is shown in Fig. 2.8 for hydrogen, helium, and nitrogen.

The figure demonstrates that the minimum achievable plate heights are similar for the three carrier gases. Although the plate height is slightly lower for the heavy carrier gas nitrogen, the differences are marginal and the minimum for nitrogen is reached at a lower average linear gas velocity and it is fairly narrow. The minimum plate height for the lighter gases helium and most of all hydrogen are reached at higher average linear gas velocities and they are much broader. In practice, this enables shorter analysis times combined with narrow peaks (Fig. 2.9).

Table 2.3 demonstrates the contribution of the B , C_M , and C_S terms to the plate height H in dependence on the inner diameter d_c and film thickness d_f of the capillary column. For low values of both d_c and d_f , the B term is dominating while the two C terms are negligible. At a low film thickness (below 1 μm), the minimum achievable plate height H_{\min} approximately corresponds to the inner diameter of the column. The contribution of the C_S term rises with thicker films

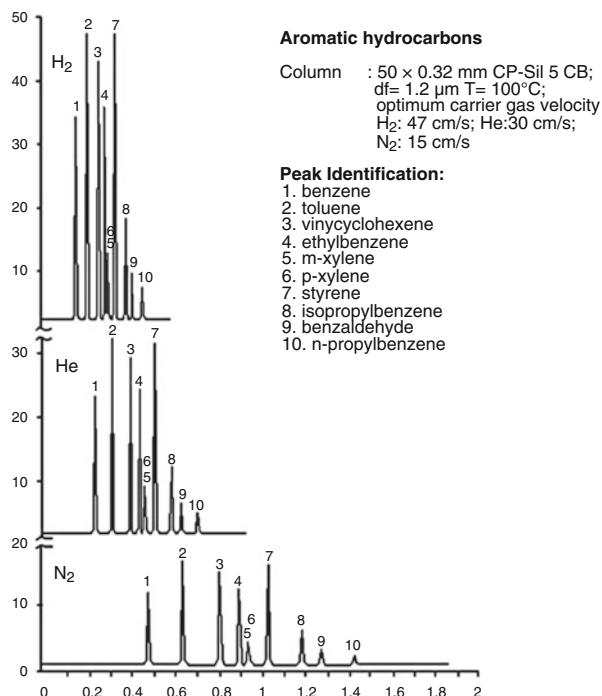


Fig. 2.9 Effect of carrier gas choice on analysis time, with permission from [11]. The chosen carrier gas velocity corresponds to \bar{u}_{opt} of the respective carrier gas.

at constant d_c and the plate height increases. For columns with large inner diameters, the percentage of the C_M term becomes significant and the influence of d_f is negligible for thin films.

2.4.4 The Optimum Carrier Gas Velocity

The H/\bar{u} plots demonstrate that both for packed and capillary columns the plate height is increased at lower average linear gas velocities by the diffusion term, while the terms of the incomplete mass transfer are dominating at higher gas velocities. An optimum average linear gas velocity \bar{u}_{opt} exists for each column. At \bar{u}_{opt} the minimum plate height (H_{min}) and consequently the maximum number of theoretical plates are obtained resulting in the highest separation efficiency and resolution.

$$\bar{u}_{\text{opt}} = \sqrt{B/C} \quad \text{and} \quad H_{\text{min}} = A + 2\sqrt{BC}$$

Table 2.3 Contribution of the B , C_M , and C_S terms for film capillary columns of different inner diameter d_c and film thickness d_f . Equations (2.34), (2.35), and (2.36) were used. Adapted from [12]

Parameters:

$$k = 5$$

$$\bar{u} = 30 \text{ cm/s}$$

$$D_G = 0.4 \text{ cm}^2/\text{s} \text{ (He)}$$

$$D_L = 1 \times 10^{-5} \text{ cm}^2/\text{s}$$

d_c	mm	0.32				0.53	
d_f	μm	0.25	0.5	1.0	5	0.5	5
B/\bar{u}	mm	0.267	0.267	0.267	0.267	0.267	0.267
$C_M \bar{u}$	mm	0.068	0.068	0.068	0.068	0.187	0.187
$C_S \bar{u}$	mm	0.002	0.007	0.028	0.694	0.007	0.694
$C \bar{u}$	mm	0.070	0.075	0.096	0.762	0.194	0.881
% $C_M \bar{u}$ on $C \bar{u}$		97.1%	90.7%	70.8%	8.9%	96.4%	21.2%
H	mm	0.34	0.34	0.36	1.03	0.46	1.15

Conclusions:

- For small d_c and d_f , B term is dominating; C terms are negligible
- H_{\min} approximately corresponds to d_c for thin films ($d_f < 1 \mu\text{m}$)
- Fraction of the C_S term increases with rising film thickness (d_c and C_M stays constant):
 - Increase of plate height

The position of the so-called Van Deemter optimum average linear gas velocity \bar{u}_{opt} depends on:

- Inner diameter of the capillary column
- Particle size of the column packing material for packed columns
- Type of mobile phase (D_G -value)
- Test solute (D_G -value, k -value)

Strictly speaking \bar{u}_{opt} is not equal for all sample components, but the differences are marginal if $k > 2$. Nevertheless, the test solute used to determine the separation efficiency (N) should always be given.

In general we should not work in the left steep branch of the H/\bar{u} curve to avoid broad peaks and long run times. Furthermore, carrier gas velocities above the efficiency optimum result in shorter run times but at the cost of reduced separation efficiency (Fig. 2.10).

Therefore, the practicing chromatographer aims for an \bar{u}_{opt} at high carrier gas velocities to obtain short run times and a minimum plate height H_{\min} that is as low as possible. In addition, the rise of the right branch of the H/\bar{u} curve should be as shallow as possible to enable higher \bar{u} values but still maintain acceptable separation efficiency. These requirements are best met by using hydrogen as carrier gas.

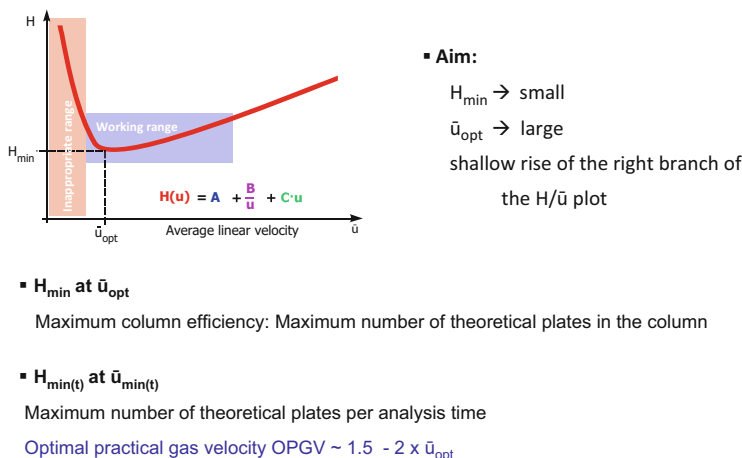


Fig. 2.10 Working range of the H/\bar{u} plot

This is especially important if the instrument is operated temperature programmed in constant pressure mode (constant column inlet pressure): The carrier gas velocity will go down with increasing column temperature, and Consequently, \bar{u}_{opt} is not maintained over the complete run and separation efficiency is lost. However, we can choose a higher carrier gas velocity ($>\bar{u}_{\text{opt}}$ in the shallow right branch of the H/\bar{u} curve) at the beginning of the run to avoid slipping in the left steep branch of the H/\bar{u} curve at the end of the run at high column temperatures, which would be combined with massive losses of separation efficiency. Nowadays, most GC separations are performed in constant flow mode ensuring that \bar{u}_{opt} is kept over the entire run.

So far, we have examined the **efficiency optimum flow (EOF)** without taking the analysis time into account, which can be fairly long. Therefore, the so-called **optimal practical gas velocity (OPGV)** was introduced [13], which specifies the maximum number of theoretical plates per analysis time. This **speed optimum flow (SOF)** is higher than the efficiency optimum flow by a factor of 1.5–2 and results an increase in plate height. Since in many cases the maximum separation efficiency of a column is not needed, but shorter run times are desired, this less elaborate approach can be used to reduce the analysis time. This is illustrated in Fig. 2.11.

At the efficiency optimum flow the lowest possible plate height is achieved, which is mainly dictated by the particle diameter for packed columns d_p respectively the inner diameter of the column d_c for capillary columns:

Packed column: $H_{\min} = 2\text{--}3 d_p$ (independent of column diameter)

Capillary column: $H_{\min} = d_c$, if $d_t \leq 0.5\text{--}1\mu\text{m}$ (independent of carrier gas)

If we determine the plate height of a non-retained solute, we obtain not only a measure for the quality of the column, but can also draw conclusions on the quality of the complete chromatographic system. By relating the plate height to the particle

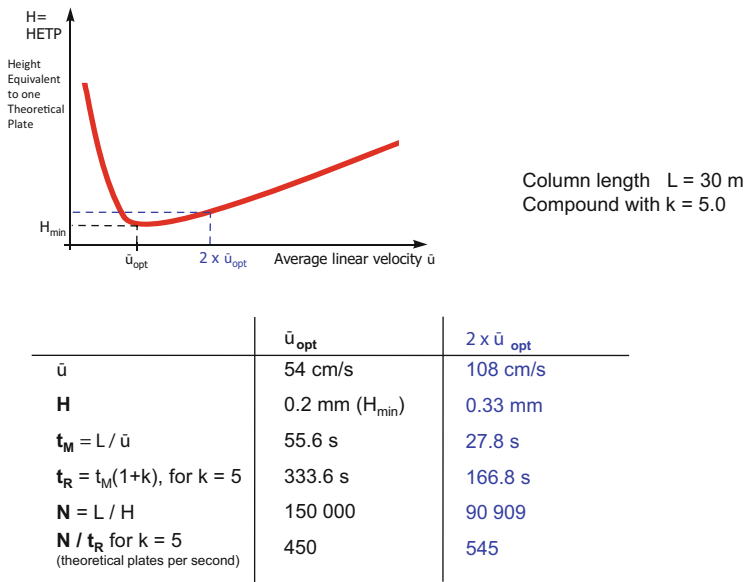


Fig. 2.11 Efficiency optimized flow (EOF) and speed optimized flow (SOF). Adapted from [14]

diameter or the column diameter, we get dimensionless (reduced) parameters initially introduced by Giddings [15, 16].

Reduced plate height:

$$h = H/d_p \quad (\text{packed column}) \tag{2.37}$$

$$h = H/d_c \quad (\text{capillary column}) \tag{2.38}$$

d_p particle diameter
 d_c column diameter

Reduced mobile phase velocity:

$$\nu = \bar{u}d_p/D_M \quad (\text{packed column}) \tag{2.39}$$

$$\nu = \bar{u}d_c/D_M \quad (\text{capillary column}) \tag{2.40}$$

where D_M is the diffusion coefficient of the analyte in the mobile phase (Alternatively, the symbol D_G is used, if the mobile phase is a gas.).

These reduced parameters proved to be beneficial in HPLC [17] and for the comparison of different types of chromatography. Thereby, the average linear velocity of the carrier gas is compared to the rate of the molecular diffusion.

2.5 Resolution

Up till now, we have only discussed the behavior of one analyte on its way through the column. However, chromatography aims to separate the components of mixtures into individual signals. The degree of separation for a adjacent peak pair is described by the resolution R_S . As for other analytical techniques, the resolution is characterized by the distance between the signals relative to the signal width (see Fig. 2.12). For peaks of similar height and without tailing the following equation applies:

$$R_S = \frac{t_{R(2)} - t_{R(1)}}{(w_{b(2)} + w_{b(1)})/2} \quad (2.41)$$

$t_{R(1)}$ retention time of the first peak

$t_{R(2)}$ retention time of the second peak

$w_{b(1)}$ peak width at the base of the first peak

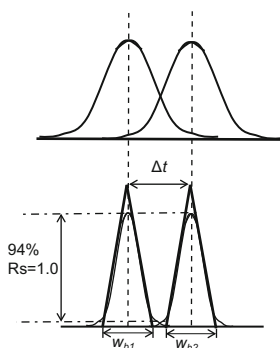
$w_{b(2)}$ peak width at the base of the second peak

The resolution can also be calculated using the peak width at half height. Assuming Gaussian peak shape with $w_b = 4\sigma$ and $w_h = 2.355\sigma$ (see Fig. 2.5), we can replace $w_b = 1.699 w_h$:

$$R_S = \frac{t_{R(2)} - t_{R(1)}}{0.845(w_{h(2)} + w_{h(1)})} = \frac{1.18(t_{R(2)} - t_{R(1)})}{(w_{h(2)} + w_{h(1)})} \quad (2.42)$$

If the peak widths of the two adjacent peaks are similar, as often observed, the following simplified equation can be used:

$$R_S \sim \frac{\Delta t}{w_{b(2)}} \quad (2.43)$$



$$R_S = \frac{t_{R(2)} - t_{R(1)}}{(w_{b2} + w_{b1})/2}$$

$$R_S \sim \frac{\Delta t}{w_{b2}}$$

$$\Delta t = t_{R(2)} - t_{R(1)}$$

Distance between the peak maxima

$t_{R(1)}$ Retention time of the first peak

$t_{R(2)}$ Retention time of the second peak

w_{b1} Peak width at the base of the first peak

w_{b2} Peak width at the base of the second peak

Fig. 2.12 Definition of chromatographic resolution

Obviously, higher R_S values correspond to a higher distance of the two adjacent peaks. The resolution can also be expressed using the standard deviation of the peak sigma (σ). At $R_S = 1.0$ the distance of the peak maxima is equivalent to the peak width at the base of the second peak, which equals 4σ . Such a separation is called a *4 sigma separation*. Peaks of similar height are about 94 % separated at a $R_S = 1.0$. For a quantitative analysis an R_S value of 1.5 is aspired, which corresponds to a *6 sigma separation*. Peaks of similar height without tailing are completely separated (base line separation) at $R_S = 1.5$. However, a higher resolution is required if small peaks adjacent to a large peak or asymmetric peaks have to be quantitatively analyzed.

2.5.1 The Resolution Equation

The definition of resolution [Eq. (2.41), Fig. 2.12] shows two general options to increase the resolution of an incompletely separated peak pair. Either the peak width is reduced by improvement of the column efficiency or the distance between the peaks is increased by increasing the selectivity. A detailed description of the interplay between column efficiency and selectivity is given by the so-called resolution equation:

$$R_S = \frac{\sqrt{N}}{4} \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k_2}{k_2 + 1} \right) \quad (2.44)$$

N plate number

α separation factor (selectivity)

k_2 retention factor of the second peak

The most important conclusions derived from this fundamental equation can be illustrated using a few examples for N , α , and k and the resulting terms of the equation (Fig. 2.13).

Efficiency term The plate number $N = L/H$ can be increased using longer columns, but resolution only improves with the square root of N . Concomitantly, the column head pressure and the analysis time increase with increasing column length. The plate height can only be reduced down to H_{\min} (efficiency optimum).

Separation/selectivity term Already small changes of α have a strong influence on the resolution. Alpha can be influenced by changes in column temperature or by selecting a different stationary phase. In contrast to liquid chromatography, where the selection of a different mobile phase also influences the selectivity, the use of a different carrier gas in GC does not affect selectivity. However, we have to keep in mind that changing the selectivity to improve the separation of a critical peak pair

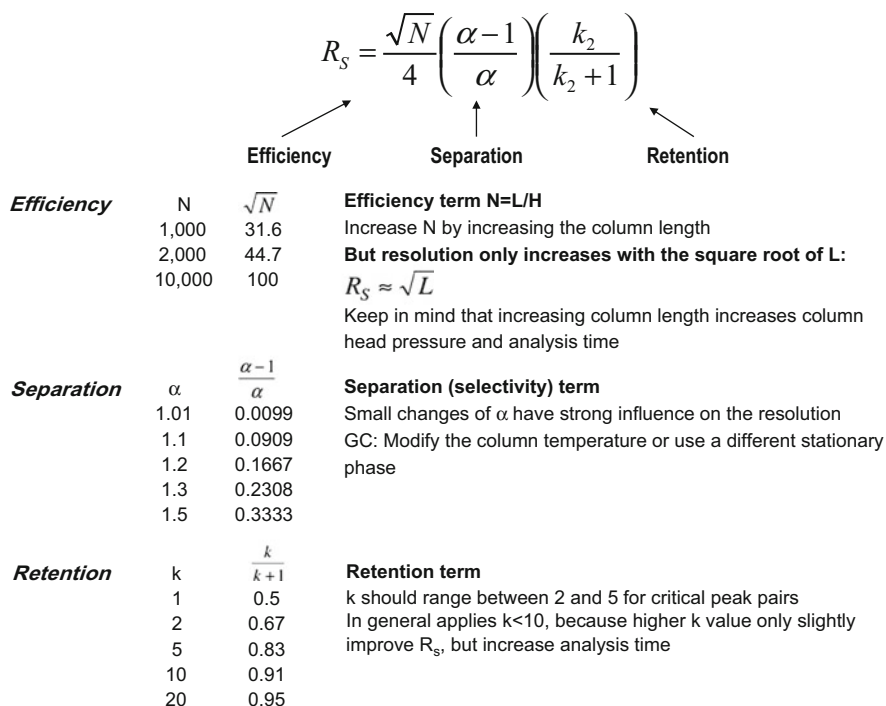


Fig. 2.13 Influence of efficiency, selectivity, and retention on resolution

might impair the separation of different peak pair in another region of the chromatogram in case of complex mixtures.

Retention term The position of a critical peak pair in the chromatogram also influences its resolution. A separation is difficult at small retention factors. The optimal retention range for a critical peak pair is between k values of 2–5. Higher values of k do not significantly improve resolution but result only in unreasonable extension of the analysis time.

If we rearrange the resolution equation to N , we can calculate the plate number and consequently the column length and analysis time needed to baseline separate a given peak pair:

$$N_{\text{req}} = 16R_s^2 \left(\frac{\alpha}{\alpha - 1} \right)^2 \left(\frac{k_2 + 1}{k_2} \right)^2 \quad (2.45)$$

2.6 Separation Number and Peak Capacity

A number of additional parameters can be used to characterize column performance. A useful concept for multicomponent analysis is to evaluate the number of peaks that can be separated with a defined resolution in a given range of the

chromatogram or the whole chromatogram. The effective peak number (EPN), the separation number (SN), and the peak capacity (n_c) can be used.

The separation number SN was introduced by R. E. Kaiser in 1962 [18]. Often the abbreviation TZ from the German expression Trennzahl is used. The separation number describes the number of peaks that can be separated between two consecutive n -alkane with carbon atom number z and $z+1$ with sufficient resolution ($R_S = 1.177$):

$$SN = \frac{t_{R(z+1)} - t_{R(z)}}{w_{h(z)} + w_{h(z+1)}} \quad (2.46)$$

$t_{R(z)}$	retention time of the n -alkane with z carbon atoms
$t_{R(z+1)}$	retention time of the n -alkane with $z+1$ carbon atoms
$w_{h(z)}$	peak width at half height of the n -alkane with z carbon atoms
$w_{h(z+1)}$	peak width at half height of the n -alkane with $z+1$ carbon atoms

Since SN depends on the n -alkanes used, they should always be specified when discussing SN. SN can be used both for isothermal and programmed temperature GC, which presents a great advantage. Furthermore, the separation number is easily derived if a retention index system (Kovats, linear retention indices) using n -alkanes is used to characterize retention (see Chap. 7).

A similar expression, the effective peak number (EPN), was proposed by Hurrell and Perry about the same time [19]. It also uses the resolution of two consecutive n -alkanes to evaluate column efficiency, but employs the peak width at the base for its calculation:

$$EPN = \frac{2(t_{R(z+1)} - t_{R(z)})}{w_{b(z)} + w_{b(z+1)}} - 1 \quad (2.47)$$

$w_{b(z)}$	peak width at base of the n -alkane with z carbon atoms
$w_{b(z+1)}$	peak width at base of the n -alkane with $z+1$ carbon atoms

SN and EPN can be transformed into each other [20, 21]:

$$EPN = 1.177 SN + 0.177 \quad (2.48)$$

In 1967 Giddings introduced the concept of peak capacity n_c [22]. It is defined as the maximum number of peaks that can be separated on a given column with a defined resolution in defined retention time window, e.g., starting from the first peak (hold-up time) up to the last peak (retention time or retention factor of the last peak). This concept is illustrated in Fig. 2.14. Obviously, peak capacity strongly depends on the peak width and therewith on column efficiency.

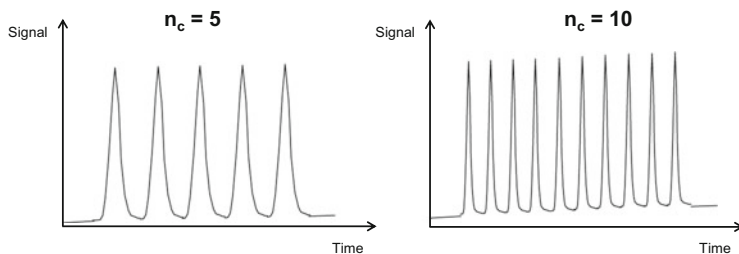


Fig. 2.14 Graphical representation of the peak capacity

If the plate number is constant for all analytes under isothermal conditions, meaning the peak width increases proportional with the retention time, n_c is calculated as follows:

$$n_c = 1 + \frac{\sqrt{N}}{4R_s} \times \ln\left(\frac{t_{R(\max)}}{t_M}\right) \quad (2.49)$$

N	plate number
R_s	resolution
t_M	hold-up time
$t_{R(\max)}$	retention time of the last peak

Example: How many peaks can be separated in 5 min with a resolution of 1 on a column with a plate number of 10,000 ($t_M = 1$ min):

$$n_c = 1 + \frac{\sqrt{10,000}}{4} \times \ln\left(\frac{5}{1}\right) = 41$$

The concept of peak capacity can also be applied to programmed temperature GC. If the peak width is constant over the complete run, the following equation can be used:

$$n_c = \frac{t_{R(\max)} - t_M}{\bar{w}} \quad (2.50)$$

t_M	hold-up time
$t_{R(\max)}$	retention time of the last peak
\bar{w}	average peak width (4σ criterion)

However, we have to keep in mind that peak capacity as well as SN/EPN are theoretical values. The peak capacity assumes that the peaks are evenly distributed across the chromatogram, which unfortunately never happens in reality. Davis and

Giddings demonstrated that peak resolution is already affected if the number of solutes exceeds 37 % of the peak capacity [23].

2.7 Evaluation of Peak Symmetry

The chromatographic theory assumes symmetrical peaks with a Gaussian shape, but in reality, often asymmetric peaks are observed due to different reasons. For example, column overloading in partition chromatography results in a shallow frontal slope of the peak, which is called fronting. Adsorption of analyte molecules at active sites results in a shallow rear edge of the peak, which is called tailing. The extent of peak asymmetry and peak tailing can be expressed either by the asymmetry factor A_S or by the tailing factor TF. The asymmetry factor is defined as ratio of the peak half-widths of rear side and the front side of the chromatographic peak measured at 10 % of the peak height (see Fig. 2.15):

$$A_S = b/a \quad (2.51)$$

a front half-width measured at 10 % of the peak height

b back half-width measured at 10 % of the peak height

A value of $A_S = 1$ represents a symmetric peak, $A_S > 1$ indicates a tailing peak, and a value < 1 is a fronting or leading peak. Small deviations from the Gaussian shape ($0.9 < A_S < 1.2$) can be mostly neglected and in the real sample analysis even A_S values of about 1.5 are often still acceptable. However, if asymmetry factors are greater than 2.0, something is wrong and the problem must be addressed.

In the pharmaceutical industry the tailing factor TF is used according to the United States Pharmacopeia (USP) [24]:

$$TF = (a + b)/2a \quad (2.52)$$

a front half-width measured at 5 % of the peak height

b back half-width measured at 5 % of the peak height

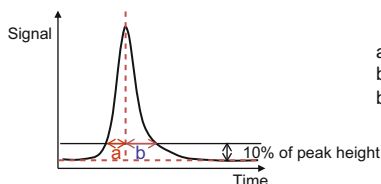
For values of less than 2.0, A_S and TF are about the same [25] and it does not matter which one is used unless it is stipulated by regulatory guidelines.

Peak shape

- Ideally, symmetric peaks with a Gaussian profile are obtained.
- In reality often asymmetric peaks occur:
 - shallow frontal slope – **Fronting**
 - shallow rear slope – **Tailing**

Asymmetry- or Tailing factor:

$$A_S = b/a$$



$a = b$ symmetric peaks
 $b > a$ Tailing
 $b < a$ Fronting

$A_S = 1$
 $A_S > 1$
 $A_S < 1$

Tailing factor according USP (US Pharmacopeia)

$$TF = (a+b)/2a$$

(a and b at 5% of peak height)

Fig. 2.15 Definition of asymmetry factor and tailing factor

2.8 Gas Flow Rate, Diffusion, Permeability, and Pressure Drop

As mentioned above, helium, hydrogen, and nitrogen are used as mobile phase (carrier gas) in gas chromatography. By applying an inlet pressure, the carrier gas is passed through the column. The flow through the column can be characterized either by the *flow rate* F (mL/min) (gas volume passed through the column per time unit) or by the *linear gas velocity* u (cm/s).

The flow rate is often measured at the column outlet using, for example, a digital flow meter. If a soap bubble flow meter is used at room temperature the measured value has to be corrected by the water vapor partial pressure and calculated for the column temperature:

$$F_a = F(1 - p_w/p_a) \quad (2.53)$$

$$F_c = F_a(T_c/T_a) \quad (2.54)$$

F measured flow rate
 F_a flow rate at ambient temperature
 F_c flow rate at column temperature
 p_a ambient pressure
 p_w partial pressure of water vapor
 T_a ambient temperature
 T_c column temperature

The *linear gas velocity at the column outlet* u_o is calculated from the flow rate F and the column cross-sectional area. In case of packed columns the interparticle porosity of the packing must be considered:

$$u_o = F(\varepsilon A_c) \quad (2.55)$$

F flow rate
 A_c cross-sectional area of the column
 ε interparticle porosity

Using the Hagen–Poiseuille equation u_o can be calculated for capillary columns from the column dimensions, carrier gas viscosity at column temperature, and the column inlet and outlet pressure:

$$u_o = \frac{d_c^2 p_o}{64\eta L} (P^2 - 1) = \frac{r_c^2 p_o}{16\eta L} (P^2 - 1) \quad (2.56)$$

d_c inner column diameter
 r_c inner column radius
 η viscosity of the carrier gas at column temperature
 L column length
 P relative pressure, $P = p_i/p_o$
 p_i absolute inlet pressure (note: Most GC instruments do not show p_i , but the pressure difference $\Delta p = p_i - p_o$. In that case the atmospheric pressure p_o has to be added to the displayed value.)
 p_o outlet pressure

2.8.1 Average Linear Velocity

On its way through the column the gas pressure drops from column inlet to the column outlet. Since $p \cdot v = \text{constant}$, the carrier gas velocity increases. Therefore, the gas pressure and the velocity are different at each point in the column. At the inlet the gas velocity is the lowest. Due to the compressibility of gases, the pressure drop across the column and the increase in gas velocity are not linear in contrast to liquid chromatography. In order to account for the compressibility of gases, the average linear velocity \bar{u} is employed, which we have already used in Sect. 2.4.2 to discuss peak broadening. The average linear velocity is derived from u_o and a correction factor for the gas compressibility j that was already introduced by James

and Martin in their fundamental publication on gas-liquid partition chromatography in 1952 [26]:

$$\bar{u} = u_o j \quad (2.57)$$

$$j = 3 (P^2 - 1) / 2 (P^3 - 1) \text{ with the relative pressure } P = p_i / p_o \quad (2.58)$$

p_i absolute column inlet pressure (see above)

p_o column outlet pressure

However, it is much easier to derive \bar{u} from the retention time of a non-retained compound t_M and the column length L :

$$\bar{u} = L / t_M \quad (2.59)$$

2.8.2 Specific Permeability

The column inlet pressure and the respective pressure drop that is needed to pass the mobile phase through the column with the required flow rate are mainly influenced by the specific permeability B_o of the column and of course the column length. B_o describes the permeability of the column for the passage of a fluid:

$$\Delta p = p_i - p_o = L \times \eta \times \bar{u} / B_o \quad (2.60)$$

L column length

η viscosity of the carrier gas at column temperature

p_i absolute inlet pressure

p_o outlet pressure

B_o specific permeability

Using a few abstractions, the specific permeability B_o was deduced for packed and capillary columns as follows:

$$\text{Packed columns : } B_o = 0.001 d_p^2 \text{ if } d_c > 10 d_p \quad (2.61)$$

$$\text{Capillary columns : } B_o = d_c^2 / 32 \quad (2.62)$$

Obviously, capillary columns have a much higher permeability than packed columns, which allows for much longer columns. The equation furthermore shows that a reduction of the particle size in packed columns or smaller inner diameters of capillary columns reduce the specific permeability requiring higher inlet pressures.

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