THIRD EDITION

CHEMICAL ANALYSIS

MODERN INSTRUMENTATION METHODS AND TECHNIQUES

FRANCIS ROUESSAC
ANNICK ROUESSAC

TRANSLATOR:
JOHN TOWEY

with website

WILEY

Chemical analysis

Chemical ANALYSIS

Modern Instrumentation Methods and Techniques

Third Edition

FRANCIS ROUESSAC
University of Le Mans
France

ANNICK ROUESSAC
University of Le Mans
France

In collaboration with
DANIEL CRUCHE and ARNAUD MARTEL

TRANSLATOR: JOHN TOWEY
With assistance from Juliet Towey
Vista Translations LLC, Portland, OR
USA

WILEY

Contents

Foreword

This book is intended to provide basic knowledge regarding the most common methods encountered in qualitative, quantitative, and structural chemical analysis. These methods are used in fields as varied as the chemical, pharmaceutical, and food industries, as well as environmental issues and various other regulations.

The methods reviewed in this book are classified as *separation methods*, *spectral methods* or *other methods*. Each of these is examined, firstly with a focus on basic concepts and then with a look at the main corresponding instrumental techniques. The book is illustrated with explanatory diagrams, drawings, and photographs, many of which are inspired from real instruments and documents obtained from manufacturers. To keep this book to a reasonable size, methods that are rarely used or have fallen into disuse are not discussed.

Clearly written, this text is addressed to a wide range of students in technical colleges (chemistry, physical measurements, applied biology, etc.) or advanced technician courses or even to students in Bachelor's and Master's programmes who wish to complement or review basic knowledge initially learned in a more fragmented manner. This book should also be useful for continuing education students and for technicians working in industry who are faced with problems of chemical analysis or who want to prepare for competitive examinations. The need for chemical analysis in many industries that had not previously used such methods, combined with the growing array of available techniques and instruments, is a further reason for many people to learn or refresh their knowledge about these techniques.

The knowledge required to approach this book corresponds to that of first-year students at undergraduate level. Hence, the authors have limited themselves to reviewing fundamental principles and taken into consideration the varying levels of students' knowledge about physical phenomena and mathematics. The text includes some theoretical reviews about the phenomena in question, in order not to lose some of the intended readers. Any readers who want more in-depth treatment of a subject may then read more specialized works, after having acquired a solid overview of the current methods and their practical aspects from this book.

It may seem like a challenge to present over 20 methods, with exercises (and their answers), in about 500 pages. That is why the authors have chosen to limit themselves to the presentation of the tools, rather than describing everything those tools can do. Only the methods have a universal character. Applications have thus been selected for purposes of illustration only. The final chapter of this book deals with basic statistical considerations.

This book originates from the lectures and laboratory work offered to IUT (technical college) students in Le Mans. This new edition has been updated and enhanced with respect to the previous ones. The third and fifth editions of this publication were translated into English under the title *Chemical Analysis, Modern Instrumentation Methods and Techniques*, John Wiley & Sons Ltd. (Chichester, 2002 and 2007). The fifth edition was translated into Spanish. Its title is *Anàlisis Quimico. Methodos y Técnicas Instrumentales Modernas*, McGraw-Hill Interamericana de Espana, S.A.U (2003). The sixth edition was translated into Korean (Dunod, 2009).

We would like to thank Daniel Cruché, Professor Emeritus, who collaborated in the proofreading of certain chapters and helped in writing some sections in this new edition. We would also like to thank all the French and foreign companies we contacted, who always kindly answered our requests to provide practical information. Their help was precious, since the field of analytical instrumentation is constantly integrating technological advances from a great variety of areas.

Lastly, we also thank our editorial team at Dunod Editions, with whom it was a pleasure to work, and more specifically, Laetitia Hérin, who launched this new edition, and Johan Dillar for his work on the production of this book.

The authors also express their gratitude to the late Professor Guy Ourisson, former President of the French Academy of Sciences, who followed the evolution of this publication throughout its consecutive editions beginning in 1992. He also gave us the great honour of writing the preface to our earliest editions. Finally, we would like to express our deepest gratitude to the late Professor Férey, member of the French Academy of Sciences and CNRS gold medal winner, for his kind preface to the seventh edition in which he announced the forthcoming arrival of the eighth edition!

Le Mans, February 2019   F. Rouessac & A. Rouessac

Nonexhaustive list of companies which kindly accepted to provide information and documents, some of which are reproduced in this book:

AB-Sciex, Agilent Technologies, American Gas & Chemical Co., American Stress Technologies, Amptek Inc, Analytik Jena, ATI, Bio-Rad, Bosch, Bruker, Camag, Chrompack, Desaga, Dionex, Edinburgh Instruments, EG&G Ortec, ETP Scientific Inc., Eurolabo, Foxboro, Graseby-Electronics, Hamamatsu, Hamilton, Hellma—Analytics, Hitachi, HORIBA-Jobin-Yvon, Imaging & Sensing Technology Corp., Inficon, JEOL, Jenway, Kratos Analytical, Leeman Labs., Leybold, Malvern Panalytical, Merck, Metorex, Metrohm, Mettler-Toledo, Microsensor Technology, Ocean Optics, Oriel, Ortec, Oxford Instruments, Perkin-Elmer, PE-Sciex, Pharmacia-Biotech, Philips, Photovac, Pike Technologies, Rheodyne, Rigaku, RTI, Safas SA, ScienTec, Servomex, Shimadzu, Siemens, Specac, Starna Scientific Ltd., Supelco, Tekmar, Teledyne Technologies, Thermo Fisher Scientific, Thermo Jarrell Ash, Torion Technologies, Tosohaas, VG Instruments, Vidac, Waters, Wilmad-LabGlass, Wyatt Technology.

About the companion website

This book is accompanied by a companion website.

www.wiley.com/go/Rouessac/Analysis3e

This website includes:
- Figures of the book

Introduction

Analytical chemistry is a close cousin to physical chemistry. It relates to the study of the chemical and physical behaviour of pure compounds or compounds in solution subject to various conditions.

It is often viewed solely in its applied aspect, with the purpose of identifying, characterizing, and quantifying chemical substances as well as developing the methods necessary for this analysis. This reductive aspect of analytical chemistry is none other than *chemical analysis*, which is the subject of this book.

Any study of chemical analysis involves addressing varied fields of knowledge. To reach the desired goal, it calls on many concepts, including some that range very far away from chemistry, in the usual sense of the word. It is a multidisciplinary science whose repercussions are felt in all experimental sciences.

In chemical analysis, it is common to distinguish between two categories of methods. Firstly, there are chemical methods, based on a specific reaction of the *analyte* (the compound being assayed) with reagents, and then there are physical techniques, which make use of the physico-chemical properties of analytes.

This second category of methods, now in the forefront, has replaced the traditional 'wet' methods, which are very limited in their performance but which were at the origin of the field of *analytical chemistry*. Most of this book is therefore dedicated to modern techniques, which have been greatly developed over these past few decades with the rise of miniaturized sensors, digitization, and computing power. These improvements have thus enabled the use of very efficient mathematical tools and less bulky equipment. As an example, we can compare the imposing Raman spectrophotometers from the 1960s with the portable Raman spectrometers that are now used for quality control in numerous fields.

And so was born instrumental analysis with its incredible arsenal of processes and devices which we now often find installed outside of traditional analytical laboratories.

The evolution of technologies has led to the creation of very efficient instruments that open up new possibilities. This is especially true with the introduction of coupled methods, nondestructive testing, and the concept of *speciation* (distinction between the various structures in which an element may be present in the sample). These methods use small samples that require little or no preparation prior to measurement. Current analytical trends also include an increase in miniaturized, portable instruments, as well as specific or automatic analysers.

In general, the basic principle consists in measuring a physical value, followed by establishing a simple relationship between this measurement – the device's

response – and the composition of the analyte in the test sample. The search for this relationship often consists in a mathematical linearization between the device's response and the analyte concentration. This relationship may be simple and direct (the response is proportional to concentration), or it may be more complex.

Whatever the tool used to establish the relationship between the signal and the concentration, we also have to determine the limits of quantification. Below that limit, any attempt at quantification is bound to fail, while above it the relationship no longer applies.

To conduct these studies, analysts must not only be trained in the various techniques, but they must also know the basic concepts of chemistry, particularly since a compound can often be assayed by various methods. Choosing a good method, and if possible the best method, requires knowing many parameters. Therefore, when a new analytical objective has been defined, the problem must be addressed methodically. We must first *choose the right method*: spectroscopic, electrochemical, separation, etc. Then the *choice of technique* is made, followed by the *choice of process* in terms of sampling and prior treatment of the sample. Finally, a *protocol*, the recipe so to speak, is established. In general, this is a process governed by established national or international standards, which leads to the chosen operating procedure. This approach focuses on standardizing all the steps: from sample preparation all the way to conducting measurements. Finally, results must be established according to existing standards. Additionally, the raw analytical data conserved as computer files cannot be changed. This entire approach is the subject of official texts known as Good Laboratory Practice (GLP).

To recommend the best method in order to resolve an analytical problem, there is a science called *chemometrics*. Its purpose is to help the analyst, as a function of specific requirements and aims: minimum sampling plan, appropriate methodology, data processing and interpretation of results. Thanks to the use of IT tools, it seeks to provide a correct answer by exploiting the results with statistical methods in order to reduce the number of tests for long or costly analyses.

In this way, users may acquire devices that meet the standard of precision and quality necessary to acquire certification or to have a laboratory officially recognized for the quality of its results. These accreditation procedures are now imposed by many control organizations around the world.

Analytical chemistry is therefore essential in many fields other than the traditional ones of chemistry or chemical engineering. It can be found in fields as varied as medicine, food, biochemistry, the environment (pollution), safety (explosives, drugs and chemicals), and art. Now more widely used in human activities than ever, analytical chemistry can be of benefit to us all.

Chapter 1
General Aspects of Chromatography

INTRODUCTION

Chromatography, the process by which the components of a mixture can be separated, has become one of the primary analytical methods for the identification and quantification of compounds. The basic principle is founded on the concentration equilibrium of the components of interest between two immiscible phases. One is called the stationary phase, because it is immobilized within a column or fixed upon a support, while the second, called the mobile phase, is forced through the first. The phases are chosen such that the components of the sample have differing solubilities in each phase. The differential migration of compounds leads to their separation. This hydrodynamic process, which has been constantly evolving since its discovery, is an analytical method that no laboratory involved in molecular analysis can ignore, as its applications are so numerous.

1.1 GENERAL CONCEPTS OF ANALYTICAL CHROMATOGRAPHY

Chromatography is a physico-chemical method of separation of components within mixtures, liquid or gaseous, in the same vein as distillation, crystallization or fractionated extraction. The applications of this process are therefore potentially numerous, since many heterogeneous mixtures, or those in solid form, can be dissolved by a suitable solvent (which becomes, of course, a supplementary component of the mixture).

A basic chromatographic process may be described as (Figure 1.1):

1. A vertical, hollow glass tube (the *column*) is filled with a suitable finely powdered solid, the *stationary phase*.

2. At the top of this column is placed a small volume of the *sample* to be separated into individual components.

3. The sample is then forced through the column from inlet to outlet by continuous addition of the *mobile phase*, carrying the various constituents of the sample along with it. If the components migrate at different velocities, they will become separated from each other and can be recovered, each in solution with the mobile phase.

While this use of chromatography has continued since its origins, this process became a method of analysis with the idea of measuring the retention time of compounds through the column in order to identify them. To do so, it became essential to control certain parameters (flow rate, temperature, etc.) and a detector had to be placed at the column's outlet to identify compositional changes in the mobile phase. This form of chromatography, whose goal is not simply to recover the components but to measure their retention time, has developed slowly.

The identification of a compound by chromatography is achieved by comparison. To identify a compound, which may be either A or B, using chromatography, we compare
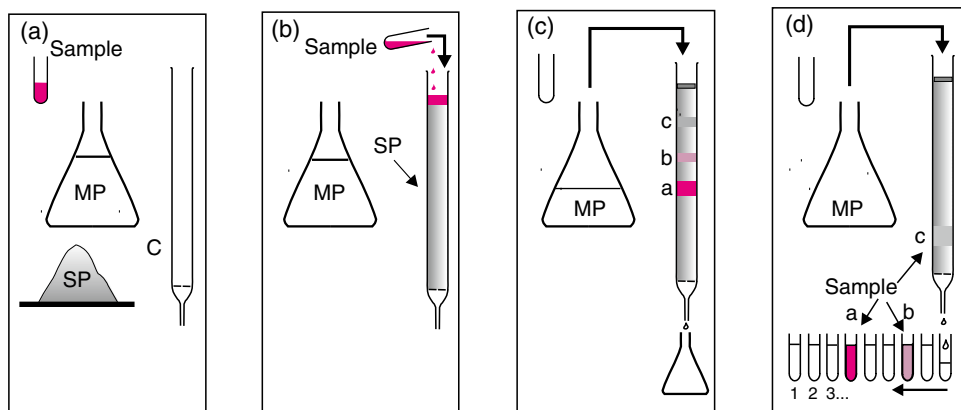
Figure 1.1 A basic experiment in chromatography. (a) The necessary ingredients (C, column; SP, stationary phase; MP, mobile phase and sample); (b) introduction of the sample; (c) start of elution; (d) recovery of the products following separation.

its *retention time* with those for the two reference compounds A and B previously recorded using the same apparatus and the same experimental conditions.

In this experiment, there was no true separation (A and B were pure products), only a comparison of the products' retention times. However, this method does have three weaknesses: the procedure is fairly slow; absolute identification is unattainable; and the physical contact between the sample and the stationary phase could modify the sample's properties, in particular the retention times.

This specific method of separation, in its modern form, was first undertaken at the beginning of the twentieth century by the botanist Mikhail Tswett (or Tsvet), who is credited with inventing the terms *chromatography* and *chromatogram*.

The technique has improved considerably since its beginnings. Nowadays, chromatographs are piloted by software programs that run highly efficient miniature columns able to separate nano-quantities of sample. These instruments comprise a complete range of accessories designed to ensure repeatability of successive experiments by the perfect control of the different parameters of separation. Thus it is possible to obtain, during successive analyses of the same sample conducted several hours apart, recordings that are reproducible to within a second (Figure 1.2).

The specific recording that is obtained for each separation is called a *chromatogram*. It corresponds to a two-dimensional diagram that reveals the variations of composition of the eluting mobile phase as it exits the column. To obtain this read-out, a sensor, or *detector*, of which there exists a great variety, needs to be placed at the outlet of the column.
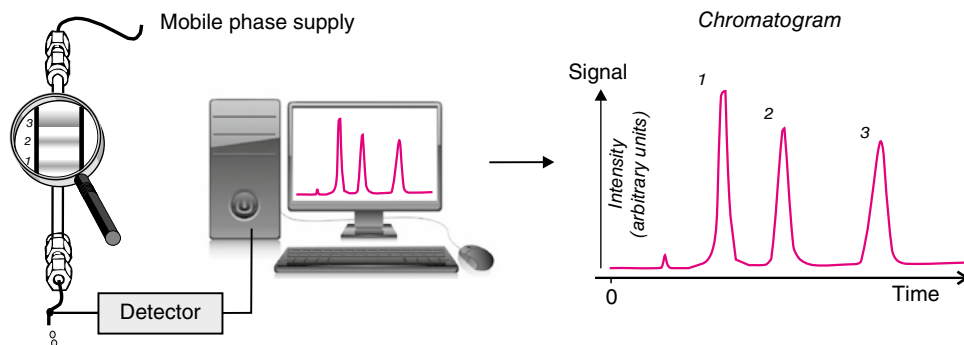
Figure 1.2 The principle of analysis by chromatography. The chromatogram, the essential graph of every chromatographic analysis, is obtained from variations, as a function of time, of an electrical signal emitted by the detector. It is either produced in real time or reconstructed at a later time from values that have been digitized and stored. The chromatography software recalculates these values and puts them in the desired format. This chromatogram illustrates the separation of a mixture of three principal components. Note that the order of appearance of the compounds corresponds to the relative position of each constituent on the column.

The identification of a molecular compound from the chromatogram can sometimes be risky. A better method consists in associating two different complementary methods, for example, a chromatograph coupled with a second instrument, such as a mass spectrometer or an infrared spectrometer. These *coupled* (or *two-dimensional*) techniques provide two independent types of information (retention times and the spectrum). Therefore, it is possible to determine without ambiguity the composition of complex mixtures or the concentration of certain compounds on the nanogram level (confirmation analyses).

1.2 THE CHROMATOGRAM

The *chromatogram* is a curve representing the variation over time of a parameter related to the concentration or quantity of the solute at the column outlet (Figure 1.3). Time (or very rarely the *elution volume*) is found on the horizontal axis, where the time origin coincides with the introduction of the sample in the injection system. The detector response is found on the vertical axis. The *baseline* corresponds to the detector response in the absence of any solute. The separation is complete between two compounds when the chromatogram shows two *chromatographic peaks* that start from and return to the baseline.

A component is characterized by its *retention time* t_R, which represents the time elapsed between sample introduction and the detection of its peak maximum on the
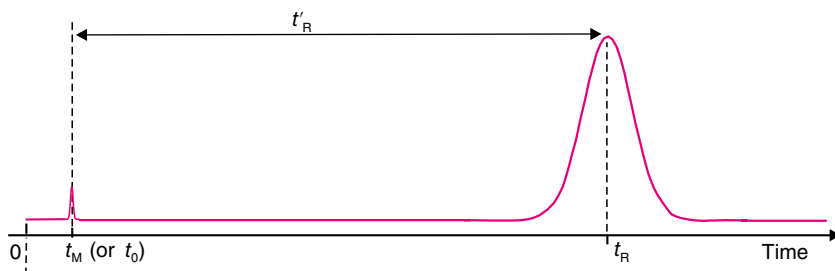
Figure 1.3 Chromatographic elution curve. Example of a graph of Eq. (1.1).

chromatogram. In an ideal case, t_R is independent of the quantity injected. The longer the retention time, the wider the peak is.

A component that is not retained will elute out of the column at time t_M, called the *hold-up time* or *dead time*[1] (also designated t_0). The difference between the retention time and the hold-up is referred to as the adjusted retention time of the compound t'$_R$.

In quantitative analysis, we often simply separate the mixture from the compound(s) to be assayed. If the signal sent by the sensor varies linearly with the concentration of a compound, then the same variation will occur for the area under the corresponding peak on the chromatogram.

1.3 GAUSSIAN PEAKS AND REAL PEAKS

On a chromatogram, the ideal elution peak would have the same form as the graphical representation of the normal distribution of random errors (Gaussian curve). In keeping with the classic notation, μ corresponds to the retention time of the eluting peak and σ to the standard deviation of the peak (σ^2 represents the *variance*). y represents the signal as a function of time x from the detector located at the outlet of the column (Figure 1.4).

This is why ideal elution peak signals of a compound are usually described by the probability density function (Eq. (1.2)).

Equation (1.1) is a mathematical relationship describing a Gaussian function, whatever the x variable. In this expression, σ represents the width unit to describe the peak and μ corresponds to the horizontal axis of the Gaussian curve (in this case, retention time t_R). If we make the peak symmetry axis correspond with the new time origin (μ or $t_R = 0$), we obtain Eq. (1.2)).

$$y = \frac{1}{\sigma\sqrt{2\pi}} \cdot \exp\left[-\frac{(x-\mu)^2}{2\sigma^2}\right] \tag{1.1}$$

---

[1] The symbols used follow IUPAC recommendations – ***Pure and Applied Chemistry***, 65(4), 819 (1993).

$$\delta = 2.35\ \sigma$$
$$\omega = 4\ \sigma$$
$$\omega = 1.7\ \delta$$
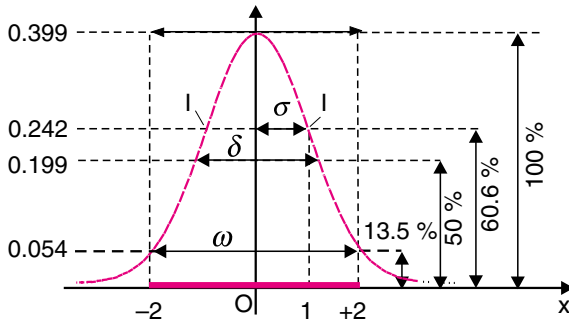
The area between –2 and +2 accounts for 95.4% of the total area under the curve and over the x-axis

Figure 1.4 Characteristics of an ideal chromatographic peak. Meaning of the three classic parameters and summary of characteristics of a Gaussian curve.

$$y = \frac{1}{\sqrt{2\pi}} \cdot \exp\left[-\frac{x^2}{2}\right] \tag{1.2}$$

This function is characterized by a symmetrical curve (maximum at $x = 0$, $y = 0.399$) possessing two inflection points at $x = \pm 1$ (Figure 1.4), whose y-value is 0.242 (i.e. 60.6% of the maximum value). The width of the curve at the inflection points is equal to 2σ ($\sigma = 1$).

In chromatography, δ represents the full width at half-maximum (FWHM, $\delta = 2.35\sigma$) and σ^2 the *variance* of the peak. The width of the peak 'at the base' is labelled ω and corresponds to the base of the triangle formed from the tangents to the inflection point *I* of the Gaussian curve. It is measured at 13.5% of the peak height. At this position, for a Gaussian curve, $\omega = 4\sigma$ by definition.

Real chromatographic peaks often deviate significantly from the ideal Gaussian form. There are several reasons for this. In particular, the peak's half-width at the inflection point is not only due to elution in the column but also to injection and detection, which we summarize with the following expression:

$$\sigma^2{}_{tot} = \sigma^2{}_{inj} + \sigma^2{}_{col} + \sigma^2{}_{det} \tag{1.3}$$

where $\sigma^2{}_{tot}$, $\sigma^2{}_{inj}$, $\sigma^2{}_{col}$, $\sigma^2{}_{det}$ are, respectively, the total variance (as observed experimentally), the variance due to *injection* (injection time, time for the sample to penetrate into the column), the variance due to the *column* (elution), and the variance due to *detection* (dead volume between column outlet and detector, detector response time, etc.).

1.4 PLATE THEORY

For more than half a century, different theories have been and continue to be proposed to model chromatography. The best known are those employing a statistical approach (stochastic theory), the theoretical plate model or a molecular dynamics approach.

To explain the mechanism of migration and separation of compounds on the column, the oldest model, known as Craig's *theoretical plate model*, is a static approach now judged to be obsolete, but which once offered a simple description of the separation of constituents.

Although chromatography is a continuous phenomenon, Craig's model considered that each solute moves progressively along a sequence of distinct static steps. This elementary process is represented by a cycle of adsorption/desorption. The continuity of these steps reproduces the migration of the compounds on the column, in a similar fashion to that achieved by an animated film, giving the illusion of movement through a sequence of fixed images. Each step corresponds to a new state of equilibrium for the *entire* column.

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

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

$$H = \frac{L}{N} \tag{1.4}$$

This approach employs the rules of polynomial expansion to calculate, for a given plate, the masses distributed between the two phases present. This theory has a major fault in that it does not take into account the dispersion in the column due to the diffusion of the compounds.

The term *theoretical plate* comes from an early approach to describe chromatography by analogy with the distillation model proposed by Martin and Synge (Nobel laureates in Chemistry, 1952). This term, though widely used in chromatography for historical reasons, has no physical significance, in contrast to its homonym which serves to measure the performance of a distillation column. It may have been better to name it after Tswett, for example!

The total retention time t_R of the solute on the column can be subdivided into two terms: t_M (hold-up time), which includes the times during which the solute is dissolved in the mobile phase and travels at the same speed as this phase, and t_S, the time during which it is attached to the stationary phase. Between two successive transfers from one phase to the other, it is accepted that the concentrations have the time to re-equilibrate.

Chromatography involves at least three sets of equilibria: solute/mobile phase, solute/stationary phase, and mobile phase/stationary phase. In a more recent theory of chromatography, it is no longer claimed that the molecules are immobilized by the stationary phase, but rather that they are simply slowed down when passing in close proximity to it.

Various physical phenomena may reveal an asymmetry in the chromatographic peak. More specifically, there is a concentration irregularity in the substance deposit zone at the column head. Moreover, the speed of the mobile phase is null on the wall and at a maximum in the centre of the column. The observed asymmetry of a peak is represented by two parameters: one named the *peak asymmetry factor* (F_a) (Eq. (1.5)), measured at 10% of the peak height, and the other named *tailing factor* (F_t) (Eq. (1.6)) measured at 5% of peak height (for the meaning of *a* and *b* in these equations, see Figure 1.5).

$$F_a = \frac{b}{a} \tag{1.5}$$

$$F_t = \frac{a+b}{2a} \tag{1.6}$$
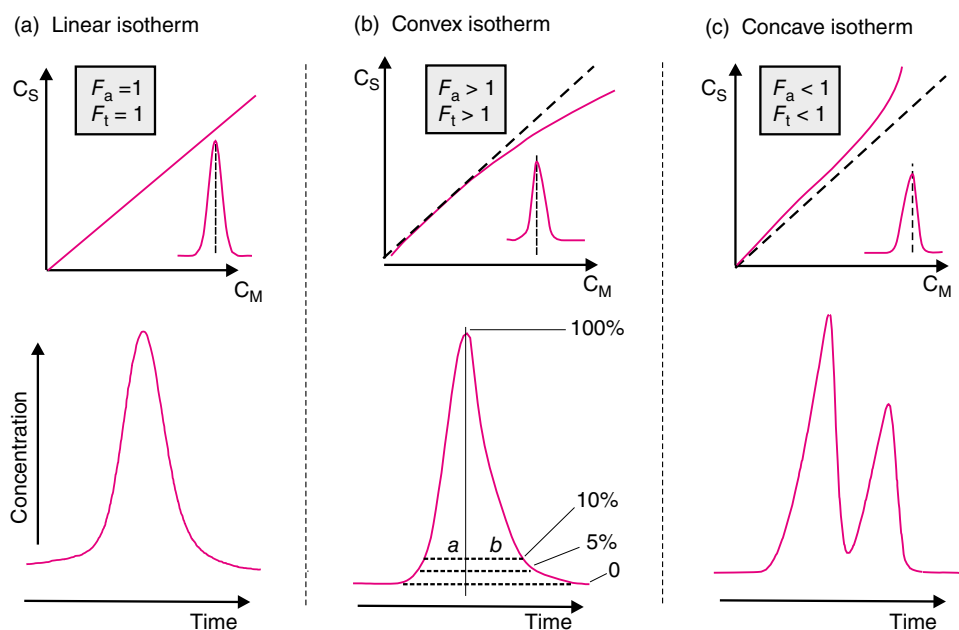
Figure 1.5 Distribution isotherms. (a) The ideal situation corresponding to the invariance of the concentration isotherm. (b) Situation in which the stationary phase is saturated – as a result, the ascent of the peak is faster than the descent. (c) The opposite situation: the component is retained too long by the stationary phase, the retention time is therefore extended, and the ascent of the peak is slower than the descent. For each type of column, the manufacturers indicate its capacity limit, expressed in ng/compound, prior to a potential deformation of the corresponding peak. The three situations are illustrated by authentic chromatograms.

1.5 NERNST PARTITION COEFFICIENT (K)

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

$$K = \frac{C_S}{C_M} = \frac{\text{Molar concentration of the solute in the stationary phase}}{\text{Molar concentration of the solute in the mobile phase}} \quad (1.7)$$

Values of K are quite variable. The higher they are, the more solute is retained. At least for liquid chromatography, they depend on the intensity of three types of interactions: stationary phase/solute, mobile phase/solute, and mobile phase/stationary phase.

Like any equilibrium constant, it is a function of temperature. It is related to variations in free enthalpy, standard enthalpy, and entropy of the exchange reaction by means of the following relationships:

$$\Delta G^0{}_T = -RT \ln K_T = \Delta H^0{}_T - T \Delta S^0{}_T \quad (1.8)$$

The experimental determination of K_T at two different temperatures enables us to calculate these variations, if we assume that the standard enthalpy and entropy variations remain virtually the same between these two temperatures. We can write:

$$\ln K_{T_1} = -\frac{\Delta H^0_T}{RT_1} + \frac{\Delta S^0_T}{R} \approx -\frac{a}{T_1} + b \quad (1.9)$$

If we know K_T at two temperatures, Eq. (1.9) helps us calculate the terms a and b.

$$a = \frac{\Delta H^0_T}{R} \quad (1.10)$$

and

$$b = \frac{\Delta S^0_T}{R} \quad (1.11)$$

In general, the standard enthalpy variation is negative. The same goes for the standard entropy variation, which corresponds to an increase in order when the solute is fixed on the stationary phase.

1.6 COLUMN EFFICIENCY

1.6.1 Theoretical Efficiency (Number of Theoretical Plates)

As the analyte migrates through the column, it occupies a continually expanding zone (Figure 1.6). This linear dispersion σ_l, measured by the variance σ_l^2, increases with the distance of migration. When this distance reaches L, the total column length, the variance will be:

$$\sigma_L^2 = H \cdot L \tag{1.12}$$

In line with the plate theory model of distillation, this approach also leads to the value of the *height equivalent to one theoretical plate H* and to the number *N* of theoretical plates ($N = L/H$).

Therefore, for any chromatogram that shows an elution peak of a compound with the temporal variance σ^2 ($\sigma = \sigma_L / \upsilon$ where υ is the mean elution speed of a solute), we can determine the *theoretical efficiency N* for this compound (Eq. (1.13)) as a function of retention time t_R and, by deduction, we can also get the value of H, knowing that $H = L/N$.

$$N = \frac{L^2}{\sigma_L^2} = \frac{L^2}{\sigma^2 \upsilon^2} = \frac{t_R^2}{\sigma^2} \tag{1.13}$$

These two parameters are indirectly accessible from the elution peak of the compound. We measure t_R and σ, whose ratio is identical to that of L over σ_L (Eq. (1.13)).
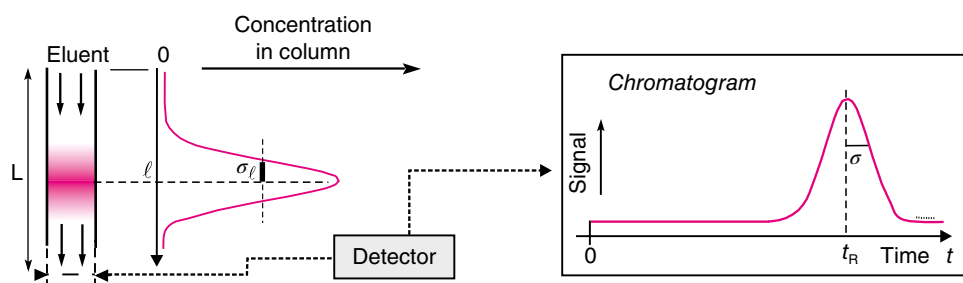
Figure 1.6 Dispersion of a solute in a column. Left, a graph corresponding to the isochronic image of the concentration of an eluted compound at a particular instant. Right, a chromatogram revealing the variation of the concentration at the column outlet, as a function of time. t_R and σ have the same ratio as L and σ_L. The efficiency N can therefore be calculated from the chromatogram by measuring σ directly. On the graph, we find about 100 theoretical plates.

On the chromatogram, σ represents the half-width of the peak at 60.6% of its height and t_R the retention time of the compound. t_R and σ should be measured in the same units (time, distance or eluted volume if the flow rate is constant). If σ is expressed in units of volume (using flow rate), then 4σ corresponds to the volume of the peak, which corresponds to the eluent volume containing 95% of the initially injected compound. By consequence of the properties of the Gaussian curve ($\omega = 4\sigma$), Eq. (1.14) results. However, because of the distortion of most peaks at their base, that equation is rarely used and Eq. (1.15) is preferred.

N is a relative parameter, since it depends upon both the solute chosen and the operating conditions adopted. Generally, a compound that appears towards the end of the chromatogram is selected in order to get a reference value, when it is unknown whether the column will successfully achieve a given separation.

$$N = 16\frac{t_R^2}{\omega^2} \tag{1.14}$$

$$N = 5.54\frac{t_R^2}{\delta^2} \tag{1.15}$$

For asymmetric peaks (which is often the case), we sometimes encounter the empirical Eq. (1.16) for calculating the column's efficiency:

$$N = 41.7\frac{\left(\dfrac{t_R}{\omega_{0.1}}\right)^2}{\left(\dfrac{a}{b}+1.25\right)} \tag{1.16}$$

where $\omega_{0.1}$ designates the width of the peak measured at 10% of its height (Figure 1.5).

1.6.2 Number of Effective Plates (Real Efficiency)

To compare the performance of columns of different design for a given compound – or to compare, in gas chromatography, the performances between a capillary column and a packed column – more realistic values are obtained by replacing the *total retention time* t_R, which appears in Eqs. (1.13)–(1.15), with the *adjusted retention time* t'_R (Figure 1.7), which does not take into account the hold-up time t_M spent by the compound in the mobile phase. The three equations then become:

$$N_{eff} = \frac{t_R'^2}{\sigma^2} \tag{1.17}$$

$$N_{eff} = 16\frac{t_R'^2}{\omega^2} \tag{1.18}$$

here,

$$k_1 = \frac{t'_{R1}}{t_M} \quad k_1 = 3.08$$

$$k_2 = \frac{t'_{R2}}{t_M} \quad k_2 = 4$$

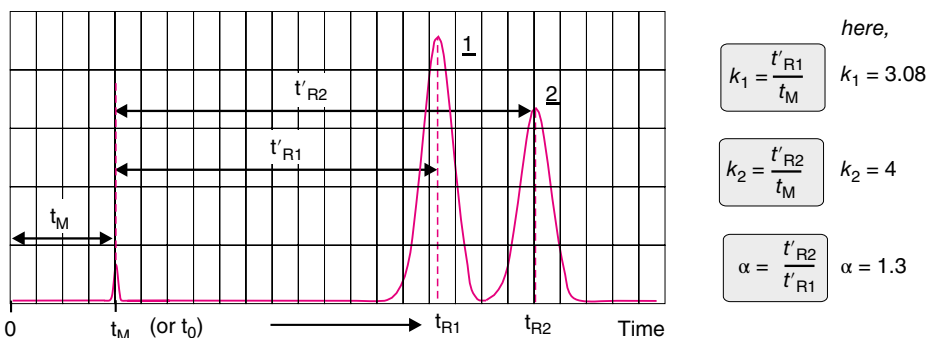$$\alpha = \frac{t'_{R2}}{t'_{R1}} \quad \alpha = 1.3$$

Figure 1.7 Retention factors and separation factor (or selectivity factor) between two adjacent compounds. Each compound has its own retention factor. On this figure, the separation factor is around 1.3. α alone is not enough to determine whether separation is really possible.

$$N_{eff} = 5.54 \frac{t'^2_R}{\delta^2} \tag{1.19}$$

Currently, it is considered that these last three equations are not very useful.

1.6.3 Plate Height

The *height equivalent to a theoretical plate H*, as already defined (Eq. (1.4)), is calculated for reference compounds to permit a comparison of columns of different lengths. However, *H* does not behave as a constant; its value depends upon the compound chosen and upon the experimental conditions.

For a long time in gas chromatography, an adjustment value called the *effective plate height H_{eff}* was calculated using the true efficiency, instead of the theoretical efficiency.

The calculation of H_{eff} from the real efficiency uses Eq. (1.20):

$$H_{eff} = \frac{L}{N_{eff}} \tag{1.20}$$

Reduced plate height

In liquid chromatography, when the column is filled with spherical particles, a parameter known as the reduced plate height *h* is often encountered. This parameter takes into account the mean diameter d_m of the particles. This eliminates the effect of the particle size for better comparison of columns with different mean diameter values. Columns with the same reduced plate height *h* will yield similar performance.

$$h = \frac{H}{d_m} = \frac{L}{N d_m} \tag{1.21}$$

1.7 RETENTION PARAMETERS

1.7.1 Retention Times

The definition of retention times has been given previously (Section 1.2).

1.7.2 Retention Volume (or Elution Volume) V_R

The *retention volume V_R* of an analyte represents the volume of mobile phase necessary to enable its migration from one end of the column to the other. To estimate this volume, different methods (direct or indirect) that depend of the physical state of the mobile phase may be used. On the chromatogram, it corresponds to the volume of mobile phase that flows through between the time of injection and the time when the peak reaches its maximum point. If the flow rate F is constant, then:

$$V_R = t_R \cdot F \tag{1.22}$$

The **volume of a peak V_{peak}** corresponds to that volume of the mobile phase in which 95% of the solute is diluted when leaving the column. It is defined by:

$$V_{peak} = \omega \cdot F \tag{1.23}$$

1.7.3 Hold-up Volume (or Dead Volume) V_M

The volume of the mobile phase in the column (known as the dead volume), V_M, corresponds to the accessible interstitial volume. It can be calculated from a chromatogram, provided a solute not retained by the stationary phase is present. The dead volume is deduced from t_M and the flow rate F:

$$V_M = t_M \cdot F \tag{1.24}$$

1.7.4 Stationary Phase Volume

This volume designated by V_s is not directly accessible from the chromatogram. In simple cases, we calculate it by subtracting the volume of the mobile phase from the total internal volume of the empty column. A column, whatever its design, may always be characterized by its *phase ratio β* defined as:

$$\beta = \frac{V_M}{V_s} \tag{1.25}$$

1.7.5 Retention (or Capacity) Factor k

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

$$k = \frac{m_S}{m_M} = \frac{C_S}{C_M} \cdot \frac{V_S}{V_M} = K\frac{V_S}{V_M} = K/\beta \tag{1.26}$$

The retention factor k, also known as the *capacity factor*, is a very important parameter in chromatography for defining column performance. Though it does not vary with the flow rate or the column length, k is not a constant, as it depends upon the experimental conditions. k is dependent via K (Nernst distribution law) on the intensity of solute–stationary phase interactions, and via β on the column's design. For this reason, it is sometimes designated by k' rather than by k alone.

This parameter takes into account the ability, great or small, of the column to retain each compound (*capacity*). When separations are being developed, k should not exceed 10. Ideally, k should be around five, otherwise the time of analysis is unduly long.

> The expression **capacity factor** presents a possible confusion with the **capacity** of a column, which is the maximum solute mass the column may retain without being saturated. This factor is given by manufacturers when describing a column.

An experimental approach to the retention factor *k*

On the basis of Craig's model, each molecule is considered as passing alternatively from the mobile phase (in which it progresses down the column) to the stationary phase (in which it is immobilized). The average speed of the progression down the column is slowed if the time periods spent in the stationary phase are long. Extrapolate now to a case that supposes n molecules of this same compound (a sample of mass m_T). If we accept that, at each instant, the ratio of the n_S molecules fixed upon the stationary phase (mass m_S) and of the n_M molecules present in the mobile phase (mass m_M) is the same as that of the times (t_S and t_M) spent in each phase for a single molecule, the three ratios will therefore have the same value:

$$\frac{n_S}{n_M} = \frac{m_S}{m_M} = \frac{t_S}{t_M} = k$$

Take the case of a molecule that spends 75% of its time in the stationary phase. Its average speed will be four times slower than if it stayed permanently in the mobile phase. As a consequence, if 4 μg of such a compound has been introduced onto the column, there will be an average of 1 μg at all times in the mobile phase and 3 μg in the stationary phase.

Since the retention time of a compound t_R is such that $t_R = t_M + t_S$, the value of k is therefore accessible from the chromatogram $\left(t_S = t'_R\right)$ (Figure 1.7):

$$k = \frac{t'_R}{t_M} = \frac{t_R - t_M}{t_M} \qquad (1.27)$$

This important relation can also be written:

$$t_R = t_M\left(1 + k\right) \qquad (1.28)$$

In light of Eqs. (1.16) and (1.18), the retention volume V_R of a solute can be written:

$$V_R = V_M\left(1 + k\right) \qquad (1.29)$$

or

$$V_R = V_M + KV_S \qquad (1.30)$$

This last expression linking the experimental parameters to the thermodynamic coefficient of distribution K is valid for ideal chromatography.

1.8 SEPARATION (OR SELECTIVITY) FACTOR

The separation factor α (Eq. (1.31)) enables the comparison of two adjacent peaks 1 and 2 present in the same chromatogram (Figure 1.7). It is defined by Eqs. (1.31) and (1.32).

By definition α is greater than unity:

$$\alpha = \frac{t'_{R(2)}}{t'_{R(1)}} \qquad (1.31)$$

or

$$\alpha = \frac{k_2}{k_1} = \frac{K_2}{K_1} \qquad (1.32)$$

The expression, connecting α to the Nernst distribution coefficients of the two solutes, shows that selectivity is dependent only on the value of these constants (intensity of interactions, temperatures) and does not depend on the column's geometry (length and diameter) or its packing (diameter of particles and quantity of stationary phase). For nonadjacent peaks, the *relative retention factor r* is calculated in a similar manner to α, and cannot be less than 1.

1.9 RESOLUTION FACTOR

To quantify the separation between two compounds, the *resolution factor R* is calculated from the chromatogram (Figure 1.8):

$$R = 2\frac{t_{R(2)} - t_{R(1)}}{\omega_1 + \omega_2} \tag{1.33}$$

For two adjacent peaks, this relationship involves two parameters: firstly, the difference between their retention times, $t_{R(2)} - t_{R(1)}$, which corresponds to the distance between the two peaks, and, secondly, their half-width at the base, ½ ($\omega_2 + \omega_1$) if we assume that each peak corresponds to an isosceles triangle (Figure 1.8).

Other expressions derived from the preceding ones and established with a view to replacing one parameter by another or to accommodating simplifications may also be employed to express the resolution. Therefore, Eqs. (1.34)–(1.36) are used quite often.

Equation (1.35) shows how resolution is affected by the efficiency, capacity factor, and selectivity factor. The chromatograms in Figure 1.9 present an experimental verification of this.

$$R = 1.177\frac{t_{R(2)} - t_{R(1)}}{\delta_1 + \delta_2} \tag{1.34}$$

$$R = \frac{1}{4}\sqrt{N_2} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k_2}{1 + k_2} \tag{1.35}$$

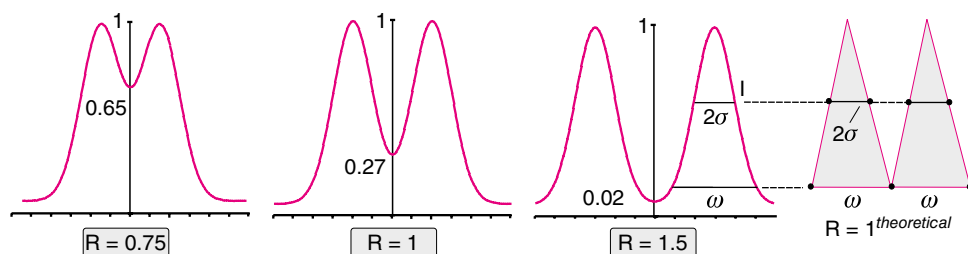$$R = \frac{\sqrt{N}}{2} \cdot \frac{k_2 - k_1}{k_1 + k_2 + 2} \tag{1.36}$$

Figure 1.8 Resolution factor. A simulation of chromatographic peaks by juxtaposition of two identical Gaussian curves to a greater or lesser extent. The visual aspects corresponding to the values of R are indicated on the diagrams. From a value of R = 1.5 the peaks can be considered to be baseline resolved, the valley between them being around 2%.

1.10 INFLUENCE OF SPEED OF THE MOBILE PHASE

In all of the previous discussion and particularly in the various equations that characterize separations, the velocity (a function of flow rate) of the mobile phase in the column is not taken into account. However, if it becomes too high, the speed, which has an influence upon the progression of the analytes down the column, disturbs the equilibrium kinetics (Solute)$_{MP}$ / (Solute)$_{SP}$, and hence it acts on their dispersion, in other words on the quality of the analysis undertaken (compare Figure 1.9).

The influence of the speed of the mobile phase was demonstrated in the case of packed columns in gas chromatography and then modelled by Van Deemter, who proposed the first kinetic equation.
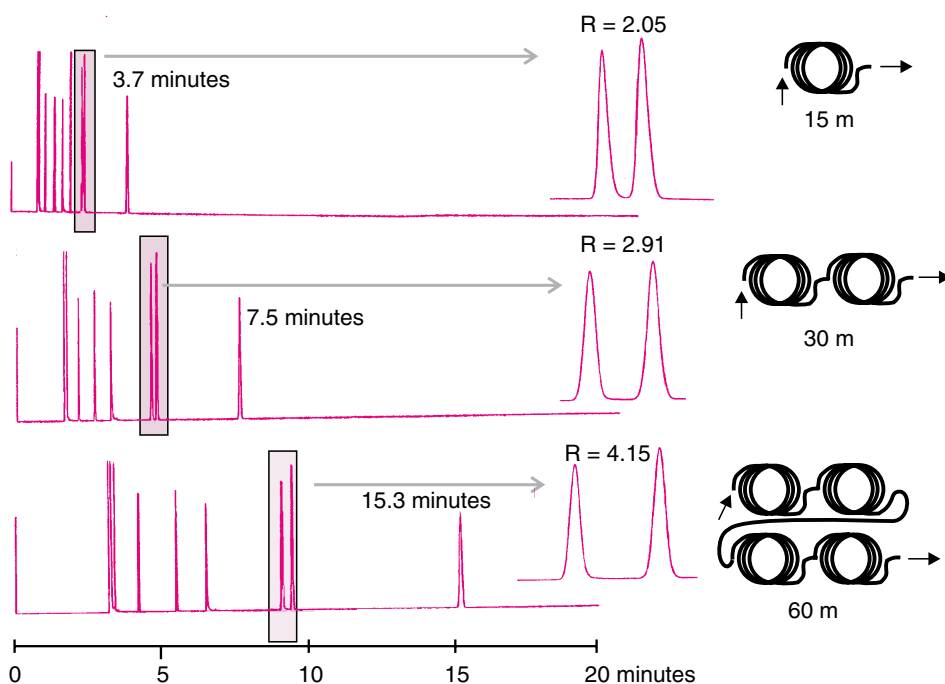

Figure 1.9 Effect of column length on the resolution. Chromatograms obtained with a GC instrument illustrating that by doubling the length of the capillary column, the resolution is multiplied by a factor of 1.41. (Source: Adapted from a document provided by the Waters company.)

1.10.1 Van Deemter Equation

The simplified equation proposed by Van Deemter in 1956, is well known for packed GC columns (Eq. (1.37)). The expression links the plate height H (HETP) to the average linear velocity of the mobile phase $\bar{u}$ in the column (Figure 1.10):

$$H = A + \frac{B}{\bar{u}} + C\bar{u} \tag{1.37}$$

This equation reveals that there exists an *optimal flow rate* for each column, corresponding to the minimum value of H, as shown by the curve of this equation. The loss in efficiency as the flow rate increases is obvious and represents what occurs when an attempt is made to rush the chromatographic separation by increasing the mobile phase flow rate. However, the loss in efficiency that occurs when the flow rate is too slow is less intuitive. To explain this phenomenon, the origins of the terms A, B, and C must be reviewed. Each of these parameters represents a domain of influence that can be perceived on the graph (Figure 1.10).

The three basic experimental coefficients A, B, and C are related to diverse physico-chemical parameters of the column and to the experimental conditions. If H is expressed in cm, A will also be in cm, B in cm²/s and C in s (where velocity is measured in cm/s). The curve of the Van Deemter equation is a hyperbola that goes through a minimum (H_{min}) when:

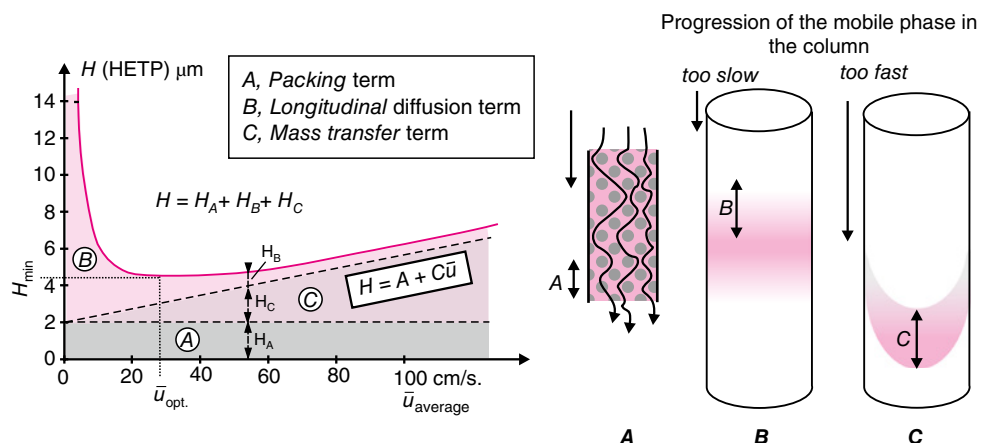$$\bar{u}_{opt} = \sqrt{\frac{B}{C}} \tag{1.38}$$

Figure 1.10 Van Deemter curve in gas chromatography with the domains of parameters A, B, and C indicated. There exists an equation similar to Van Deemter's that considers temperature: $H = A + B/T + CT$.