Encyclopedia of Separation Science

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Foreword

Separation science was first recognized as a distinct area of physical and analytical chemistry in the 1960s. The term was first coined, I believe, by the late J. Calvin Giddings, Research Professor at the University of Utah. Calvin Giddings recognized that the same basic physical principles governed a wide range of separation techniques, and that much could be learnt by applying our understanding of one such technique to others. This was especially true for his first loves, chromatography and electrophoresis and latterly field flow fractionation. Of course there are many separation techniques other than chromatography, many with a history at least as long, or indeed longer, than that of chromatography: distillation, crystallization, centrifugation, extraction, flotation and particle separation, spring to mind. Other separation techniques have emerged more recently: affinity separations, membrane separations and mass spectrometry. Most people, a few years ago, would not have classed mass spectrometry as a separation technique at all. However, with modern ionization methods, which minimize fragmentation, mixtures of compounds can first of all be separated and then each component identified through fragmentation by secondary ion-molecule collisions and further mass spectrometry. With the scale of mass spectrometry now matching that of microseparation methods such as capillary electrophoresis and capillary electrochromatography, combinations of orthogonal methods can now provide extremely powerful separation and identification platforms for characterizing complex mixtures.

Basically, all separation techniques rely on thermodynamic differences between components to discriminate one component from another, while kinetic factors determine the speed at which separation can be achieved. This applies most obviously to distillation, chromatography and electrophoresis, but is also obvious in most of the other techniques; even particle size separation by sieving can be classified in this way. The thermodynamic aspect is, of course, trivial being represented by the different sizes of the particles, as indeed it is for the size exclusion chromatography of polymers. However, the kinetic aspects are far from trivial. Anyone who has tried to sieve particles will have asked the question: is it better to fill the sieve nearly to the top and sieve for a long time, or is it better to dribble the material slowly into the sieve and just remove the heavies from time to time? One might further ask: how does one devise a continuous sieving process where large particles emerge from one port of the equipment, and small ones emerge from the other port? And how does one optimize throughput and minimize unit cost?

The publication of this *Encyclopedia of Separation Science* is a landmark for this area of science at the start of the third millennium. It will undoubtedly be of enormous value to practitioners of separation science looking for an overview and for guidance as to which method to select for a new problem, as well as to those who are at an early stage, simply dipping their toes into the waters, and trying to find out just what it is all about. Most important of all, by providing a comprehensive picture, it advances the whole field of separation science and stimulates further work on its development and application. The publishers, their editors and their authors are to be congratulated on a splendid effort.

John H. Knox
Edinburgh
8 March 2000

Preface

The ability to perform separations for the analysis, concentration or isolation of substances present in mixtures (of varying degrees of complexity) is arguably fundamental to the maintenance of our technological civilization. Separations can also be rather difficult to define, and over the course of a number of debates where we tried to 'separate the wheat from the chaff', we defined separations for the purposes of this work as '*processes of any scale by which the components of a mixture are separated from each other without substantial chemical modification*'. Of course some of the processes that are used in separations have a very long history, and the terms used to describe them are in widespread general use. So, we talk about how it is possible to distil wisdom, precipitate an argument, extract meaning and crystallize an idea and who can predict the future uses of the word chromatography? Whilst separations have been practised as an *art* for millennia, the last hundred years or so has seen the elucidation of the fundamentals that lie behind many of these processes. Thus, although separations are most widely used for achieving some practical objective, a firm theoretical understanding has been put into place that does allow the use of the term *separation science*. We have tried here to reflect the theoretical and practical aspects of the topics in this encyclopedia, and have attempted to achieve a blend of theory, practice and applications that will enable someone knowledgeable in a field to go directly to a relevant article; whilst the novice can begin with an overview and gradually iterate towards the practical application.

One thing is clear, separations cover such a wide range of topics that no single individual can be knowledgeable, let alone expert, in them all. It is against this background that we decided that an encyclopedia designed to cover this science would be of value as a single source of reference that would provide access to the whole field of separations.

For the purposes of defining the scope and coverage of the encyclopedia we have divided the area of separations into 12 families, or topic areas, using separation principles based on affinity, centrifugation, chromatography, crystallization, distillation, electrophoresis, extraction, flotation, ion exchange, mass spectrometry, membranes and particle size. Whilst there is no doubt that different editors might have grouped these slightly differently, they did not seem to us to be capable of further reduction. Taken together, we believe that they provide coverage of the whole field.

In preparing this multi-author and multi-volume work, all of the editors have been conscious of the gaps in their own expertise and the debt which they and the publishers owe to the Editorial Advisory Board, who to a large degree have compensated for the deficiencies in our own knowledge. Their help has been invaluable, as without them we would not have been able to achieve the necessary balance and it was, therefore, a source of particular sadness that one of them, Ted Woodburn, died prior to publication. Without Ted it is quite clear that the topic area of flotation would not have been so well covered, and we would like to think that he would have been well pleased with the finished work. We also hope that the masterly overview which he contributed to the encyclopedia, will be a lasting memorial to him. We are grateful to Jan Cilliers for stepping into the role of Editorial Board Advisor on flotation at short notice. We would also like to acknowledge the valuable input of G. J. Arkenbout at the beginning of this project, who was able to work with us for a short period of time before his death.

Assembling this knowledgeable and enthusiastic group of experts was a difficult task, and the editors would also wish to acknowledge the role of the Major Reference Works development team at Academic Press in this whole area, as well as thanking them for their assistance and patience throughout the project, from the initial planning to its final publication.

Finally, of course, we must acknowledge the contributions of the authors whose expertise constitutes this encyclopedia. Some of them have become firm friends in the period between the inception of this project and its completion. After all, separation science separates things but brings people together.

Ian D. Wilson, Edward R. Adlard, Michael Cooke, Colin F. Poole
Editors

Stepwise Pyrolysis

A pyrolysis in which the sample temperature is raised stepwise. The pyrolysis products are recorded between each step.

Tar

A liquid pyrolysis residue.

Temperature-Programmed Pyrolysis

A pyrolysis during which the sample is heated at a controlled rate within a temperature range in which pyrolysis occurs.

Temperature Rise Time (TRT)

The time required for a pyrolyser temperature to be increased from its initial to its final temperature.

Temperature Time Profile (TTP)

A graphical representation of temperature *versus* time for a particular pyrolysis experiment or pyrolyser.

Total heating time (THT)

The time between the onset and conclusion of the sample heating in a pyrolysis experiment.

Volatile Pyrolyzate

That portion of the pyrolystate which has adequate vapour pressure to reach the detector.

List of Symbols

$T_{(\mathrm{f,Py})}$	Final pyrolysis temperature
$T_{(\mathrm{max,Py})}$	Maximum pyrolysis temperature

Index of Acronyms

Py-GC	Pyrolysis-gas chromatography
Py-GC-IR	Pyrolysis-gas chromatography-infrared spectroscopy
Py-GC-MS	Pyrolysis-gas chromatography-mass spectrometry
Py-IR	Pyrolysis-infrared spectroscopy
Py-MS	Pyrolysis-mass spectrometry
THT	Total heating time
TRT	Temperature rise time
TTP	Temperature time profile

12A. NOMENCLATURE

Chromatography (IUPAC Recommendations 1993)

Prepared for publication by

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Abstract

This report presents definitions of terms and symbols used in all chromatographic separations. The reports covers gas, liquid, size-exclusion, ion-exchange and supercritical-fluid chromatography and both column and planar modes of separation. Definitions are included for the description of the separation process, the chromatographic system and equipment and the properties of detectors.

Introduction

The Commission on Analytical Nomenclature of IUPAC has been active for a long time in establishing nomenclatures for chromatography. After proposing suitable nomenclatures for gas chromatography [1–2] and ion exchange [3–4] the Commission developed a unified nomenclature for chromatography [5–6]. Parallel to these activities other standardization bodies and scientists have also dealt with nomenclatures on gas chromatography [7–15], supercritical-fluid chromatography [16], liquid chromatography [17–20], exclusion chromatography [21–23] and planar chromatography [24].

The original activities of the IUPAC Commission on Analytical Nomenclature aimed to create a unified nomenclature applicable to all forms of chromatography, took place over 20 years ago. Since that time chromatographic techniques have advanced significantly. Based on these developments it was decided to prepare a new, up-to-date universal chromatography nomenclature, which also considers the recommendations incorporated in the various other nomenclatures developed since the original work of IUPAC.

The present nomenclature was prepared by Dr. L. S. Ettre originally for the Commission on Analytical Nomenclature. Following the reorganization of the Commissions of the Analytical Division at the General Assembly in Lund in 1989, this project became the responsibility of the Commission on Chromatography and Other Analytical Separations (LLTC). The Nomenclature considers all the previous nomenclatures referenced above as well as the four publications dealing with these nomenclatures [25–27].

The present nomenclature deals with all chromatographic terms and definitions used in the major chromatographic techniques such as gas, liquid and supercritical-fluid chromatography, column and planar chromatography, partition, adsorption, ion-exchange and exclusion chromatography. However, it does not include terms related to the results calculated from chromatography data such as e.g. the various molecular weight terms computed from the primary data obtained by exclusion chromatography. Also it does not deal with detailed information related to detection and detectors or the relationships between chemical structure and chromatographic retention.

General Rules

In developing the unified nomenclature the rules and recommendations set up by IUPAC's Division of Physical Chemistry [28] were followed. According to these, the following symbols should be used for major physical and physico-chemical quantities and units:

area	A
density	ρ
diameter	d
diffusion coefficient	D
equilibrium constant	K
mass (weight)	W
pressure	p or P
radius	r
rate constant	k
temperature (kelvin)	T
time	t
velocity	u
viscosity	η
volume	V

The only deviation from the rules set by the Division of Physical Chemistry of IUPAC is the use of L (instead of l) for length. The reason for this is the easy interchangeability in a printed, and particularly typed, text of the

letter *l* with the numeral 'one'. Additional basic symbols accepted were *F* for the volumetric flow rates and *w* for the peak widths. Also, differentiation has been made between *p* (for pressures) and *P* (for relative pressure).

In addition to these basic rules the following additional rules are followed in the present proposal:

(a) Except for a few superscripts further differentiation is always made by using subscripts and never composite symbols.
(b) Superscripts are used for various retention times and volumes and to specifically indicate data obtained in programmed-temperature conditions.
(c) Subscripts referring to the physical conditions or the phase are capitalized, e.g. M and S for the mobile and stationary phases respectively, or, in gas chromatography, G for the gas and L for the liquid phase. Thus, e.g. the diffusion coefficient in the mobile phase is D_M and not D_m.
(d) In addition to those mentioned above, a few capitalized subscripts are used such as R for 'retention' (as in t_R and V_R), N for 'net' (as in t_N and V_N) and *F* in R_F, the retardation factor used in planar chromatography.
(e) Compound subscripts are avoided. If a given compound is indicated and there is already a subscript, and if the compound is characterized by more than a simple number or letter, then the new subscript should be in parentheses. Thus, while it is t_{Ri}, it should be $t_{R(st)}$ or $t_{R(z+1)}$.
(f) In addition to reference to the outlet of a column, subscript 'o' is also used in a number of terms to describe some fundamental values. Similarly subscript 'i' has various meanings, depending on the term in which it is used.
(g) Physical parts of the system are generally characterized by lower-case subscripts such as, c for column, p for particles or pores, and f for film.

Three tables follow the nomenclature, listing alphabetically the terms, symbols and acronyms included in the text.

Contents

1. General Terminology
1.1. Basic Definitions
1.2. Principal Methods
1.3. Classification According to the Shape of the Chromatographic Bed
1.4. Classification According to the Physical State of the Mobile Phase
1.5. Classification According to the Mechanism of Separation
1.6. Special Techniques

2. Terms Related to the Chromatographic System
2.1. Apparatus in Column Chromatography
2.2. Apparatus in Planar Chromatography

3. Terms Related to the Chromatographic Process and the Theory of Chromatography
3.1. The Chromatographic Medium
3.2. The Column
3.3. The Chromatogram
3.4. Diffusion
3.5. Temperatures
3.6. The Mobile Phase
3.7. Retention Parameters in Column Chromatography
3.8. Retention Parameters in Planar Chromatography
3.9. Distribution Constants
3.10. Terms Expressing the Efficiency of Separation

4. Terms Related to Detection
4.1. Classification of Detectors
4.2. Detector Response

4.3. Noise and Drift
4.4. Minimum Detectability
4.5. Linear and Dynamic Ranges

5. Special Terminology Used in Ion-exchange Chromatography
5.1. Basic Definitions
5.2. The Mobile Phase
5.3. The Chromatographic Medium
5.4. Capacity Values
5.5. Diffusion, Selectivity and Separation
5.6. Distribution Constants

6. Special Terminology Used in Exclusion Chromatography
6.1. The Column
6.2. Retention Parameters
6.3. Efficiency Terms

Tables
1. Index of Terms
2. List of Symbols
3. List of Acronyms Used in Chromatography

Figures
1. Typical Chromatograms
2. Typical Plane Chromatogram
3. Widths of the Gaussian Peak at Various Heights as a Function of the Standard Deviation of the Peak
4. Measurement of the Noise and Drift of a Chromatographic Detector
5. Linearity Plot of a Chromatographic Detector
6. Determination of the Linear and Dynamic Ranges of a Chromatographic Detector
7. Retention Characteristics in Exclusion Chromatography

1. General Terminology

1.1. Basic Definitions

1.1.01. Chromatography Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction.

1.1.02. Chromatogram A graphical or other presentation of detector response, concentration of analyte in the effluent or other quantity used as a measure of effluent concentration versus effluent volume or time. In planar chromatography "chromatogram" may refer to the paper or layer with the separated zones.

1.1.03. Chromatograph (verb) To separate by chromatography.

1.1.04. Chromatograph (noun) The assembly of apparatus for carrying out chromatographic separation.

1.1.05. Stationary phase The stationary phase is one of the two phases forming a chromatographic system. It may be a solid, a gel or a liquid. If a liquid, it may be distributed on a solid. This solid may or may not contribute to the separation process. The liquid may also be chemically bonded to the solid (*Bonded Phase*) or immobilized onto it (*Immobilized Phase*).

The expression *Chromatographic Bed* or *Sorbent* may be used as a general term to denote any of the different forms in which the stationary phase is used.

Note: Particularly in gas chromatography where the stationary phase is most often a liquid, the term *Liquid Phase* is used for it as compared to the *Gas Phase*, i.e. the mobile phase. However, particularly in the

early development of liquid chromatography, the term 'liquid phase' had also been used to characterize the mobile phase as compared to the 'solid phase' i.e. the stationary phase. Due to this ambiguity, the use of the term 'liquid phase' is discouraged. If the physical state of the stationary phase is to be expressed, the use of the adjective forms such as *Liquid Stationary Phase* and *Solid Stationary Phase*, *Bonded Phase* or *Immobilized Phase* is proposed.

1.1.05.1. Bonded phase A stationary phase which is covalently bonded to the support particles or to the inside wall of the column tubing.

1.1.05.2. Immobilized phase A stationary phase which is immobilized on the support particles, or on the inner wall of the column tubing, e.g. by *in situ* polymerization (cross-linking) after coating.

1.1.06. Mobile phase A fluid which percolates through or along the stationary bed, in a definite direction. It may be a liquid (*Liquid Chromatography*) or a gas (*Gas Chromatography*) or a supercritical fluid (*Supercritical-Fluid Chromatography*). In gas chromatography the expression *Carrier Gas* may be used for the mobile phase. In elution chromatography the expression *Eluent* is also used for the mobile phase.

1.1.07. Elute (verb) To chromatograph by elution chromatograph. The process of elution may be stopped while all the sample components are still on the chromatographic bed or continued until the components have left the chromatographic bed.

Note: The term 'elute' is preferred to the term *Develop* used in former nomenclatures of planar chromatography.

1.1.08. Effluent The mobile phase leaving the column.

1.1.09. Sample The mixture consisting of a number of components the separation of which is attempted on the chromatographic bed as they are carried or eluted by the mobile phase.

1.1.10. Sample components The chemically pure constituents of the sample. They may be unretained (i.e. not delayed) by the stationary phase, partially retained (i.e. eluted at different times) or retained permanently. The terms *Elute* or *Analyte* are also acceptable for a sample component.

1.1.11. Solute A term referring to the sample components in partition chromatography.

1.1.12. Solvent A term sometimes referring to the liquid stationary phase in partition chromatography.

Note: In liquid chromatography the term 'solvent' has been often used for the mobile phase. This usage is not recommended.

1.1.13. Zone A region in the chromatographic bed where one or more components of the sample are located. The term *Band* may also be used for it.

1.2. Principal Methods

1.2.01. Frontal chromatography A procedure in which the sample (liquid or gas) is fed continuously into the chromatographic bed. In frontal chromatography no additional mobile phase is used.

1.2.02. Displacement chromatography A procedure in which the mobile phase contains a compound (*the Displacer*) more strongly retained than the components of the sample under examination. The sample is fed into the system as a finite slug.

1.2.03. Elution chromatography A procedure in which the mobile phase is continuously passed through or along the chromatographic bed and the sample is fed into the system as a finite slug.

1.3. Classification According to the Shape of the Chromatographic Bed

1.3.01. Column chromatography A separation technique in which the stationary bed is within a tube. The particles of the solid stationary phase or the support coated with a liquid stationary phase may fill the whole inside volume of the tube (*Packed Column*) or be concentrated on or along the inside tube wall leaving an open, unrestricted path for the mobile phase in the middle part of the tube (*Open-Tubular Column*).

1.3.02. Planar chromatography A separation technique in which the stationary phase is present as or on a plane. The plane can be a paper, serving as such or impregnated by a substance as the stationary bed (*Paper Chromatography*, PC) or a layer of solid particles spread on a support, e.g. a glass plate (*Thin-Layer Chromatography*, TLC). Sometimes planar chromatography is also termed *Open-Bed Chromatography*.

1.4. Classification According to the Physical State of the Mobile Phase

1.4.01. Chromatographic techniques are often classified by specifying the physical state of *both* phases used. Accordingly, the following terms are in use:

Gas-liquid chromatography (GLC)
Gas-solid chromatography (GSC)
Liquid-Liquid chromatography (LLC)
Liquid-solid chromatography (LSC)

The term *Gas-Liquid Partition Chromatography* (GLPC) can also be found in the literature. However, often distinction between these modes is not easy. For example, in GC, a liquid may be used to modify an adsorbent-type solid stationary phase.

1.4.02. Gas chromatography (GC) A separation technique in which the mobile phase is a gas. Gas chromatography is always carried out in a column.

1.4.03. Liquid chromatography (LC) A separation technique in which the mobile phase is a liquid. Liquid chromatography can be carried out either in a column or on a plane.

Note: Present-day liquid chromatography generally utilizing very small particles and a relatively high inlet pressure is often characterized by the term *High-Performance* (or *High-Pressure*) *Liquid Chromatography*, and the acronym HPLC.

1.4.04. Supercritical-fluid chromatography (SFC) A separation technique in which the mobile phase is a fluid above and relatively close to its critical temperature and pressure.

Note: In general the terms and definitions used in gas or liquid chromatography are equally applicable to supercritical-fluid chromatography.

1.5. Classification According to the Mechanism of Separation

1.5.01. Adsorption chromatography Separation is based mainly on differences between the adsorption affinities of the sample components for the surface of an active solid.

1.5.02. Partition chromatography Separation is based mainly on differences between the solubilities of the sample components in the stationary phase (gas chromatography), or on differences between the solubilities of the components in the mobile and stationary phases (liquid chromatography).

1.5.03. Ion-exchange chromatography Separation is based mainly on differences in the ion exchange affinities of the sample components.

Note: Present day ion-exchange chromatography on small particle high efficiency columns and usually utilising conductometric or spectroscopic detectors is often referred to as *Ion Chromatography* (IC).

1.5.04. Exclusion chromatography Separation is based mainly on exclusion effects, such as differences in molecular size and/or shape or in charge. The term *Size-Exclusion Chromatography* may also be used when separation is based on molecular size. The terms *Gel Filtration* and *Gel-Permeation Chromatography* (GPC) were used earlier to describe this process when the stationary phase is a swollen gel. The term *Ion-Exclusion Chromatography* is specifically used for the separation of ions in an aqueous phase.

1.5.05. Affinity chromatography This expression characterizes the particular variant of chromatography in which the unique biological specificity of the analyte and ligand interaction is utilized for the separation.

1.6. Special Techniques

1.6.01. Reversed-phase chromatography An elution procedure used in liquid chromatography in which the mobile phase is significantly more polar then the stationary phase, e.g. a microporous silica-based material with chemically bonded alkyl chains.

Note: The term "reverse phase" is an incorrect expression to be avoided.

1.6.02. Normal-phase chromatography An elution procedure in which the stationary phase is more polar than the mobile phase. This term is used in liquid chromatography to emphasize the contrast to reversed-phase chromatography.

1.6.03. Isocratic analysis The procedure in which the composition of the mobile phase remains constant during the elution process.

1.6.04. Gradient elution The procedure in which the composition of the mobile phase is changed continuously or stepwise during the elution process.

1.6.05. Stepwise elution The elution process in which the composition of the mobile phase is changed in steps during a single chromatographic run.

1.6.06. Two-dimensional chromatography A procedure in which parts or all of the separated sample components are subjected to additional separation steps. This can be done, e.g. by conducting a particular fraction eluting from the column into another column (system) having different separation characteristics. When combined with additional separation steps, this may be described as *Multi-Dimensional Chromatography*.

In planar chromatography two-dimensional chromatography refers to the chromatographic process in which the components are caused to migrate first in one direction and subsequently in a direction at right angles to the first one; the two elutions are carried out with different eluents.

1.6.07. Isothermal chromatography A procedure in which the temperature of the column is kept constant during the separation.

1.6.08. Programmed-temperature chromatography (temperature programming) A procedure in which the temperature of the column is changed systematically during a part or the whole of the separation.

1.6.09. Programmed-flow chromatography (flow programming) A procedure in which the rate of flow of the mobile phase is changed systematically during a part or the whole of the separation.

1.6.10. Programmed-pressure chromatography (pressure programming) A procedure in which the inlet pressure of the mobile phase is changed systematically during a part or whole of the separation.

1.6.11. Reaction chromatography A technique in which the identities of the sample components are intentionally changed between sample introduction and detection. The reaction can take place upstream of the column when the chemical identity of the individual components passing through the column differs from that of the original sample, or between the column and the detector when the original sample components are separated in the column but their identity is changed prior to entering the detection device.

1.6.11.1. Pyrolysis-gas chromatography A version of reaction chromatography in which a sample is thermally decomposed to simpler fragments before entering the column.

1.6.11.2. Post-column derivatization A version of reaction chromatography in which the separated sample components eluting from the column are derivatized prior to entering the detector. The derivatization process is generally carried out "on-the-fly", i.e. during transfer of the sample components from the column to the detector. Derivatization may also be carried out before the sample enters the column or the planar medium; this is *pre-column* (*preliminary*) *derivatization*.

2. Terms Related to the Chromatographic System

2.1. Apparatus in Column Chromatography

2.1.01. Pump A device designed to deliver the mobile phase at a controlled flow-rate to the separation system. Pumps are generally used in liquid chromatography.

2.1.01.1. Syringe pumps Pumps with a piston, which advances at a controlled rate within a smooth cylinder to displace the mobile phase.

2.1.01.2. Reciprocating pumps Pumps with a single or multiple chamber, from which the mobile phase is displaced by reciprocating piston(s) or diaphragm(s).

2.1.01.3. Pneumatic pumps Pumps which employ a gas to displace the liquid mobile phase either directly or via a piston.

2.1.02. Sample injector A device by which a liquid, solid or gaseous sample is introduced into the mobile phase of the chromatographic bed.

2.1.02.1. Direct injector A device which directly introduces the sample into the mobile-phase stream.

2.1.02.2. Bypass injector A device in which the sample is first introduced into a chamber (loop), temporarily isolated from the mobile phase system by valves, which can be switched to make an instantaneous diversion of the mobile phase stream through the chamber to carry the sample to the column. A bypass injector may also be known as a *Valve Injector* or *Sampling Valve* (see 2.1.02.7).

2.1.02.3. On-column injector A device in which the sample is directly introduced into the column. In gas chromatography the on-column injector permits the introduction of the liquid sample into the column without prior evaporation.

2.1.02.4. Flash vaporizer A heated device used in gas chromatography. Here the liquid sample is introduced into the carrier gas stream with simultaneous evaporation and mixing with the carrier gas prior to entering the column.

2.1.02.5. Split injection A sample introduction technique used in gas chromatography. The sample is flash vaporized and after thorough mixing of the sample with carrier gas, the stream is split into two portions, one being conducted to the column and the other being discarded.

2.1.02.6. Programmed temperature vaporizer (PTV) A sample introduction device used in gas chromatography. The liquid sample is introduced, usually with a syringe, into a device similar to a flash vaporizer, the temperature of which is kept low, below the boiling point of the sample components. After withdrawal of the syringe, the device is heated up very rapidly in a controlled fashion to evaporate the sample into the continuously flowing carrier gas stream. The PTV may also be used in the split mode: in this case, the carrier gas stream containing the evaporated sample components is split into two portions, one of which is conducted into the column while the other is discarded.

2.1.02.7. Gas sampling valve A bypass injector permitting the introduction of a gaseous sample of a given volume into a gas chromatograph.

2.1.03. Column oven A thermostatically controlled oven containing the column, the temperature of which (*Separation Temperature* or *Column Temperature*) can be varied within a wide range.

2.1.04. Fraction collector A device for recovering fractional volumes of the column effluent.

2.1.05. Detector A device that measures the change in the composition of the eluent by measuring physical or chemical properties.

2.2. Apparatus in Planar Chromatography

2.2.01. Spotting device The syringe or micropipet used to deliver a fixed volume of sample as a spot or streak to the paper or thin-layer media at the origin.

2.2.02. Elution chamber (developing chamber) A closed container, the purpose of which is to enclose the media used as well as the mobile phase to maintain a constant environment in the vapor phase.

2.2.02.1. Sandwich chamber A chamber in which the walls are close enough to the paper or plate to provide a relatively fast equilibration.

2.2.02.2. Ascending elution (ascending development) A mode of operation in which the paper or plate is in a vertical or slanted position and the mobile phase is supplied to its lower edge; the upward movement depends on capillary action.

2.2.02.3. Horizontal elution (horizontal development) A mode of operation in which the paper or plate is in a horizontal position and the mobile-phase movement along the plane depends on capillary action.

2.2.02.4. Descending elution (descending development) A mode of operation in which the mobile phase is supplied to the upper edge of the paper or plate and the downward movement is governed mainly by gravity.

2.2.02.5. Radial elution (radial development) or circular elution (circular development) A mode of operation in which the sample is spotted at a point source at or near the middle of the plane and is carried outward in a circle by the mobile phase, also applied at that place.

2.2.02.6. Anticircular elution (anticircular development) The opposite of 2.2.02.5. Here the sample as well as the mobile phase is applied at the periphery of a circle and both move towards the center.

2.2.02.7. Chamber saturation (saturated development) This expression refers to the uniform distribution of the mobile phase vapor through the elution chamber prior to chromatography.

2.2.02.8. Unsaturated elution (unsaturated development) This expression refers to chromatography in an elution chamber without attaining chamber saturation.

2.2.02.9. Equilibration The expression refers to the level of saturation of the chromatographic bed by the mobile-phase vapor prior to chromatography.

2.2.03. Visualization chamber A device in which the planar media may be viewed under controlled-wavelength light, perhaps after spraying with chemical reagents to render the separated components as visible spots under specified conditions.

2.2.04. Densitometer A device which allows portions of the developed paper or thin-layer media to be scanned with a beam of light of a specified wavelength for measurements of UV or visible light absorption or fluorescence, providing values which can be used for the quantisation of the separated compounds.

3. Terms Related to the Chromatographic Process and the Theory of Chromatography

3.1. The Chromatographic Medium

3.1.01. Active solid A solid with sorptive properties.

3.1.02. Modified active solid An active solid the sorptive properties of which have been changed by some treatment.

3.1.03. Solid support A solid that holds the stationary phase but, ideally, does not contribute to the separation process.

3.1.04. Binders Additives used to hold the solid stationary phase to the inactive plate or sheet in thin-layer chromatography.

3.1.05. Gradient layer The chromatographic bed used in thin-layer chromatography in which there is a gradual transition in some property.

3.1.06. Impregnation The modification of the separation properties of the chromatographic bed used in planar chromatography by appropriate additives.

3.1.07. Packing The active solid, stationary liquid plus solid support, or swollen gel contained in a tube.

3.1.07.1. Totally porous packing Here the stationary phase permeates each porous particle.

3.1.07.2. Pellicular packing In this case the stationary phase forms a porous outer shell on an impermeable particle.

3.1.08. Particle diameter (d_p) The average diameter of the solid particles.

3.1.09. Pore radius (r_p) The average radius of the pores within the solid particles.

3.1.10. Liquid-phase loading A term used in partition chromatography to express the relative amount of the liquid stationary phase in the column packing. It is equal to the mass fraction (%) of liquid stationary phase in the total packing (liquid stationary phase plus support).

3.2. The Column

3.2.01. Column The tube and the stationary phase contained within, through which the mobile phase passes.

3.2.02. Packed column A tube containing a solid packing.

3.2.03. Open-tubular column A column, usually having a small diameter in which either the inner tube wall, or a liquid or active solid held stationary on the tube wall acts as the stationary phase and there is an open, unrestricted path for the mobile phase.

3.2.03.1. Wall-coated open-tubular (WCOT) column In these columns the liquid stationary phase is coated on the essentially unmodified smooth inner wall of the tube.

3.2.03.2. Porous-layer open-tubular (PLOT) column In these columns there is a porous layer on the inner wall. Porosity can be achieved by either chemical means (e.g. etching) or by the deposition of porous particles on the wall from a suspension. The porous layer may serve as a support for a liquid stationary phase or as the stationary phase itself.

3.2.03.3. Support-coated open-tubular (SCOT) column A version of a PLOT column in which the porous layer consists of support particles and was deposited from a suspension.

3.2.04. Capillary column A general term for columns having a small diameter. A capillary column may contain a packing or have the stationary phase supported on its inside wall. The former case corresponds to a *Packed Capillary Column* while the latter case corresponds to an *Open-Tubular Column*. Due to the ambiguity of this term its use without an adjective is discouraged.

3.2.05. Column volume (V_c) The geometric volume of the part of the tube that contains the packing:

$$V_c = A_c L$$

where A_c is the internal cross-sectional area of the tube and L is the length of the packed part of the column.

In the case of wall-coated open-tubular columns the column volume corresponds to the geometric volume of the whole tube having a liquid or a solid stationary phase on its wall.

3.2.06. Bed volume Synonymous with *Column Volume* for a packed column.

3.2.07. Column diameter (d_c) The inner diameter of the tubing.

3.2.08. Column radius (r_c) The inside radius of the tubing.

3.2.09. Column length (L) The length of that part of the tube which contains the stationary phase.

3.2.10. Cross-sectional area of the column (A_c) The cross-sectional area of the empty tube:

$$A_c = \pi r_c^2 = \pi (d_c/2)^2$$

3.2.11. Interparticle volume of the column (V_o) The volume occupied by the mobile phase between the particles in the packed section of a column. It is also called the *Interstitial Volume* or the *Void Volume* of the column.

3.2.11.1. In liquid chromatography, the interparticle volume is equal to the mobile-phase hold-up volume (V_M) in the ideal case, neglecting any extra-column volume.

3.2.11.2. In gas chromatography, the symbol V_G may be used for the interparticle volume of the column. In the ideal case, neglecting any extra-column volume, V_G is equal to the corrected gas hold-up volume (V_M^o) (see 3.6.03 and 3.7.04):

$$V_G = V_M^o = V_M \cdot j$$

3.2.12. Interparticle porosity (ε) The interparticle volume of a packed column per unit column volume:

$$\varepsilon = V_o/V_c$$

It is also called the *Interstitial Fraction* of the column.

3.2.13. Extra-column volume The volume between the effective injection point and the effective detection point, excluding the part of the column containing the stationary phase. It is composed of the volumes of the injector, connecting lines and detector.

3.2.13.1. Dead-volume This term is also used to express the extra-column volume. Strictly speaking, the term "dead-volume" refers to volumes within the chromatographic system which are not swept by the mobile phase. On the other hand, mobile phase is flowing through most of the extra-column volumes. Due to this ambiguity the use of the term "dead-volume" is discouraged.

3.2.14. Liquid-phase film thickness (d_f) A term used in connection with open-tubular columns to express the average thickness of the liquid stationary phase film coated on the inside wall of the tubing.

3.2.15. Stationary-phase volume (V_S) The volume of the liquid stationary phase or the active solid in the column. The volume of any solid support is not included. In the case of partition chromatography with a liquid stationary phase, it is identical to the *Liquid-Phase Volume* (V_L).

3.2.16. Mass (weight) of the stationary phase (W_S) The mass (weight) of the liquid stationary phase or the active solid in the column. The mass (weight) of any solid support is not included. In the case of partition chromatography with a liquid stationary phase it is identical to the *Liquid Phase Mass* (*Weight*) (W_L).

3.2.17. Phase ratio (β) The ratio of the volume of the mobile phase to that of the stationary phase in a column:

$$\beta = V_o/V_S$$

In the case of open-tubular columns the geometric internal volume of the tube (V_c) is to be substituted for V_o.

3.2.18. Specific permeability (B_o) A term expressing the resistance of an empty tube or packed column to the flow of a fluid (the mobile phase). In the case of a packed column

$$B_o = \frac{d_p^2 \varepsilon^3}{180(1-\varepsilon)^2} \approx \frac{d_p^2}{1000}$$

In the case of an open-tubular column

$$B_o = \frac{r_c^2}{8}$$

3.2.19. Flow resistance parameter (Φ) This term is used to compare packing density and permeability of columns packed with different particles; it is dimensionless.

$$\Phi = d_p^2/B_o$$

where d_p is the average particle diameter. In open-tubular columns $\Phi = 32$.

3.3. The Chromatogram

3.3.01. Differential chromatogram A chromatogram obtained with a differential detector (see **Figure 1A**).

3.3.02. Integral chromatogram A chromatogram obtained with an integral detector (see Figure 1B).

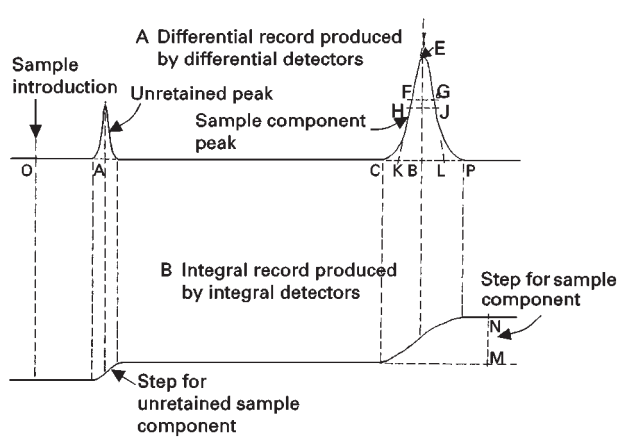

Figure 1 Typical chromatogram: A, differential record produced by differential detector; B, integral record produced by integral detector.

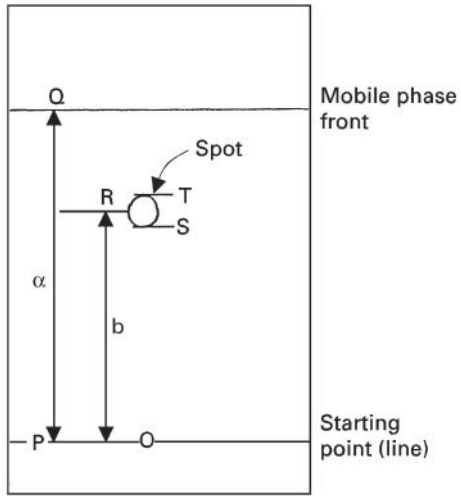

Figure 2 Typical planar chromatogram.

3.3.03. Starting point or line The point or line on a chromatographic paper or layer where the substance to be chromatographed is applied (P in **Figure 2**).

3.3.04. Spot A zone in paper and thin-layer chromatography of approximately circular appearance.

3.3.04.1. Spot diameter (ST in Figure 2) The width of the sample component spot before or after chromatography.

3.3.05. Baseline The portion of the chromatogram recording the detector response when only the mobile phase emerges from the column.

3.3.06. Peak The portion of a differential chromatogram recording the detector response when a single component is eluted from the column (see **Figure 1A**). If separation is incomplete, two or more components may be eluted as one *Unresolved Peak*.

3.3.06.1. Peak base (CP in Figure 1A) The interpolation of the baseline between the extremities of the peak.

3.3.06.2. Peak area (CHFEGJP in Figure 1A) The area enclosed between the peak and the peak base.

3.3.06.3. Peak maximum (E in Figure 1A) The point on the peak at which the distance to the peak base, measured in a direction parallel to the axis representing detector response, is a maximum.

3.3.06.4. Peak height (EB in Figure 1A) The distance between the peak maximum and the peak base, measured in a direction parallel to the axis representing detector response.

3.3.06.5. Standard deviation (σ) The term in the exponent of the equation relating the width and height of a Gaussian peak:

$$y = y_o \,.\, \exp. - \left[\frac{x^2}{2\sigma^2}\right]$$

where y is the peak height at any point on the peak, y_o is the peak height at maximum, x is the distance from the ordinate (i.e. half of the width at that point), and σ is the standard deviation of the peak. In practice, the standard deviation can be calculated from one of the peak-width values specified below.

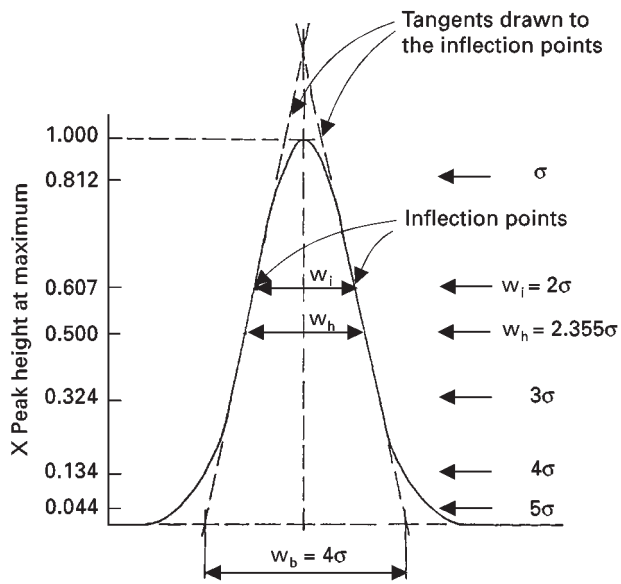

Figure 3 Widths of a Gaussian peak at various heights, as a function of the standard deviation of the peak.

3.3.06.6. Variance of the peak The square of the standard deviation (σ^2).

3.3.07. Peak-widths Peak-widths represent retention dimensions (time or volume) parallel to the baseline. If the baseline is not parallel to the axis representing time or volume, then the peak-widths are to be drawn parallel to this axis. Three peak-width values are commonly used in chromatography (see Figure 1A and **Figure 3**).

3.3.07.1. Peak-width at base (w_b) (KL in Figure 1A and Figure 3) The segment of the peak base intercepted by the tangents drawn to the inflection points on either side of the peak.

3.3.07.2. Peak-width at half height (w_h) (HJ in Figure 1A and Figure 3) The length of the line parallel to the peak base at 50% of the peak height that terminates at the intersection with the two limbs of the peak

Note: The peak-width at base (w_b) may be called the 'base width'. However, the peak width at half height (w_h) must never be called the 'half width' because that has a completely different meaning. Also, the symbol $w_{1/2}$ should never be used instead of w_h.

3.3.07.3. Peak-width at inflection points (w_i) (FG in Figure 1A and Figure 3) The length of the line drawn between the inflection points parallel to the peak base.

3.3.07.4. In the case of Gaussian (symmetrical) peaks, the peak-widths are related to the standard deviation (σ) of the peak according to the following equations:

$$w_b = 4\sigma$$

$$w_h = 2\sigma\sqrt{(2 \ln 2)} = 2.355\sigma$$

$$w_i = 2\sigma$$

3.3.08. Tailing Asymmetry of a peak such that, relative to the baseline, the front is steeper than the rear. In paper chromatography and thin-layer chromatography, it refers to the distortion of a spot showing a diffuse region behind the spot in the direction of flow.

3.3.09. Fronting Asymmetry of a peak such that, relative to the baseline, the front is less steep than the rear. In paper chromatography and thin-layer chromatography, it refers to the distortion of a spot, showing a diffuse region in front of the spot in the direction of flow.

3.3.10. Step The portion of an integral chromatogram recording the amount of a component, or the corresponding change in the signal from the detector as the component emerges from the column (see Figure 1B).

3.3.10.1. Step height (NM in Figure 1B) The distance, measured in the direction of detector response, between straight-line extensions of the baselines on both sides of a step.

3.3.11. Internal standard A compound added to a sample in known concentration to facilitate the qualitative identification and/or quantitative determination of the sample components.

3.3.12. External standard A compound present in a standard sample of known concentration and volume which is analysed separately from the unknown sample under identical conditions. It is used to facilitate the qualitative identification and/or quantitative determination of the sample components. The volume of the external standard (standard sample) need not to be known if it is identical to that of the unknown sample.

3.3.13. Marker A reference substance chromatographed with the sample to assist in identifying the components.

3.4. Diffusion

3.4.01. The diffusion coefficient (D) is the amount of a particular substance that diffuses across a unit area in 1 s under the influence of a gradient of one unit.

It is usually expressed in the units $cm^2\ s^{-1}$.

3.4.02. Diffusion coefficient in the stationary phase (D_S or D_L) The diffusion coefficient characterizing the diffusion in the stationary phase. In partition chromatography with a liquid stationary phase, the symbol D_L may be used to express this term.

3.4.03. Diffusion coefficient in the mobile phase (D_M or D_G) The diffusion coefficient characterizing the diffusion in the mobile phase. In gas chromatography where the mobile phase is a gas, the symbol D_G may be used to express this term.

3.4.04. Diffusion velocity (u_D) This term is used in liquid chromatography in the expression of the reduced mobile-phase velocity (see 3.6.05.3). The diffusion velocity expresses the speed of diffusion into the pores of the particles:

$$u_D = D_M/d_p$$

3.5. Temperatures

3.5.01. Ambient temperature (T_a) The temperature outside the chromatographic system.

3.5.02. Injection temperature The temperature within the injection device.

3.5.03. Separation temperature (T_c) The temperature of the chromatographic bed under isothermal operation. In column chromatography it is called the *Column Temperature*.

3.5.04. Temperatures during programmed-temperature analysis

3.5.04.1. Initial temperature The temperature of the chromatographic bed (column) at the start of the analysis. Temperature programming might start immediately upon sample introduction or it can be preceded by a short isothermal period (*Initial Isothermal Temperature*). In the case, the time of the *Initial Isothermal Period* must also be specified.

3.5.04.2. Program rate The rate of increase of column temperature. The rate of temperature increase is usually linear ($^{\circ}C.min^{-1}$) but it may also be non-linear. During one analysis the temperature rate may be changed and/or the temperature programming may be interrupted by an isothermal period. In this case one is speaking about *Multiple Programming*. In multiple programming each program must be specified by its initial and final temperatures and program rate.

3.5.04.3. Mid-analysis isothermal temperature The temperature of the column in an isothermal period during elution. The corresponding time (*Mid-Analysis Isothermal Period*) must also be specified.

3.5.04.4. Final temperature The highest temperature to which the column is programmed.

3.5.04.5. Final isothermal temperature The final temperature of the program if it is followed by an isothermal period. The time corresponding to the *Final Isothermal Period* must also be specified.

3.5.04.6. Retention temperature The column temperature corresponding to the peak maximum.

3.5.05. Detector temperature The temperature of the detector cell. In the case of a detector incorporating a flame, it refers to the temperature of the detector base.

3.6. The Mobile Phase

3.6.01. Mobile phase viscosity (η) The viscosity of the mobile phase at the temperature of the chromatographic bed.

3.6.02. Pressures

3.6.02.1. Inlet pressure (p_i) The *absolute* pressure at the inlet of a chromatographic column.

3.6.02.2. Outlet pressure (p_o) The absolute pressure at the exit of a chromatographic column. It is usually but not necessarily equal to the *Ambient Pressure* (p_a), the atmospheric pressure outside the chromatographic system.

3.6.02.3. Pressure drop (Δp) The difference between the inlet and outlet pressures:

$$\Delta p = p_i - p_o$$

3.6.02.4. Relative pressure (P) The ratio of the inlet and outlet pressures:

$$P = p_i/p_o$$

3.6.03. Mobile phase compressibility correction factor (j) A factor, applying to a homogeneously filled column of uniform diameter, that corrects for the compressibility of the mobile phase in the column. It is also called the *Compressibility Correction Factor*. In gas chromatography, the correction factor can be calculated as:

$$j = \frac{3}{2}\frac{p^2 - 1}{p^3 - 1} = \frac{3}{2}\frac{(p_i/p_o)^2 - 1}{(p_i/p_o)^3 - 1}$$

In liquid chromatography the compressibility of the mobile phase is negligible.

Note: In former nomenclatures the term 'pressure gradient correction factor' was sometimes used to express the same term. This is, however, an incorrect name, because it is not the pressure gradient but the compression of the mobile phase which necessitates the use of this factor. In liquid chromatography, where mobile phase compression is negligible, no correction factor has to be applied to the mobile phase velocity; however, there is still a pressure gradient along the column.

3.6.04. Flow rate The volume of mobile phase passing through the column in unit time.

3.6.04.1. The flow rate is usually measured at the column outlet, at ambient pressure (p_a) and temperature (T_a, in K); this value is indicated with the symbol F. If a water-containing flowmeter was used for the measurement (e.g. the so-called soap bubble flowmeter) then F must be corrected to dry gas conditions in order to obtain the *Mobile Phase Flow Rate at Ambient Temperature* (F_a):

$$F_a = F(1 - p_w/p_a)$$

where p_w is the partial pressure of water vapor at ambient temperature.

3.6.04.2. In order to specify chromatographic conditions in column chromatography, the flow-rate (*Mobile Phase Flow Rate at Column Temperature*, F_c) must be expressed at T_c (kelvin), the column temperature:

$$F_c = F_a(T_c/T_a)$$

3.6.05. Velocities

3.6.05.1. Mobile-phase velocity (u) The linear velocity of the mobile phase across the average cross-section of the chromatographic bed or column. It can be calculated from the column flow-rate at column temperature (F_c), the cross-sectional area of the column (A_c) and the interparticle porosity (ε):

$$u = F_c/(\varepsilon A_c)$$

In practice the mobile phase velocity is usually calculated by dividing the column length (L) by the retention time of an unretained compound (t_M; see 3.7.03):

$$u = L/t_M$$

3.6.05.2. In gas chromatography, due to the compressibility of the carrier gas, the linear velocity will be different at different longitudinal positions in the column. Therefore two terms must be distinguished:

The *Carrier Gas Velocity at the Column Outlet* (u_o) can be obtained as above, from the carrier gas flow rate measured at column outlet:

$$u_o = F_c/(\varepsilon A_c)$$

The *Average Linear Carrier Gas Velocity* ($\bar{u}$) is obtained from u_o, by correcting it for gas compressibility:

$$\bar{u} = u_o j$$

The average linear carrier gas velocity can also be obtained by dividing the column length (L) by the retention time of an unretained compound (t_M):

$$\bar{u} = L/t_M$$

In liquid chromatography where mobile phase compression is negligible, $\bar{u} = u$.

3.6.05.3. Reduced mobile phase velocity (υ) A term used mainly in liquid chromatography. It compares the mobile phase velocity with the velocity of diffusion into the pores of the particles (the so-called diffusion velocity, u_D: see 3.4.04):

$$\upsilon = \bar{u}/u_D = \bar{u}d_p/D_M$$

In open-tubular chromatography:

$$\upsilon = \bar{u}d_c/D_M$$

3.7. Retention Parameters in Column Chromatography

3.7.01. Retention parameters may be measured in terms of chart distances or times, as well as mobile phase volumes; e.g., t'_R (time) is analogous to V'_R (volume). If recorder speed is constant, the chart distances are directly proportional to the times; similarly if the flow rate is constant, the volumes are directly proportional to the times.

Note: In gas chromatography, or in any chromatography where the mobile phase expands in the column, V_M, V_R and V'_R represent volumes under column outlet pressure. If F_c, the carrier gas flow rate at the column outlet and corrected to column temperature (see 3.6.04.2), is used in calculating the retention volumes from the retention time values, these correspond to volumes at column temperatures.

3.7.02. The various conditions under which retention volumes (times) are expressed are indicated by superscripts: thus, a prime (′; as in V'_R) refers to correction for the hold-up volume (and time) while a circle (°; as in $V_R{}^\circ$) refers to correction for mobile-phase compression. In the case of the net retention volume (time) both corrections should be applied: however, in order not to confuse the symbol by the use of a double superscript, a new symbol (V_N, t_N) is used for the net retention volume (time).

3.7.03. Hold-up volume (time) (V_M, t_M) The volume of the mobile phase (or the corresponding time) required to elute a component the concentration of which in the stationary phase is negligible compared to that in the mobile phase. In other words, this component is not retained at all by the stationary phase. Thus, the hold-up volume (time) is equal to the *Retention Volume* (*Time*) *of an Unretained Compound*. The hold-up volume (time) corresponds to the distance OA in Figure 1A and it includes any volumes contributed by the sample injector, the detector, and connectors.

$$t_M = V_M/F_c$$

In gas chromatography this term is also called the *Gas Hold-up Volume* (*Time*).

3.7.04. Corrected gas hold-up (volume ($V_M{}^\circ$) The gas hold-up volume multiplied by the compression (compressibility) correction factor (j):

$$V_M{}^\circ = V_M.j$$

Assuming that the influence of extracolumn volume on V_M is negligible,

$$V_M{}^\circ = V_G$$

(see 3.2.11.2)

3.7.05. Total retention volume (time) (V_R, t_R) The volume of mobile phase entering the column between sample injection and the emergence of the peak maximum of the sample component of interest (OB in Figure 1a), or the corresponding time. It includes the hold-up volume (time):

$$t_R = V_R/F_c$$

3.7.06. Peak elution volume (time) ($\bar{V}_R$, $\bar{t}_R$) The volume of mobile phase entering the column between the start of the elution and the emergence of the peak maximum, or the corresponding time. In most of the cases, this is equal to the total retention volume (time). There are, however, cases when the elution process does not start immediately at sample introduction. For example, in liquid chromatography, sometimes the column is washed with a liquid after the application of the sample to displace certain components which are of no interest and during this treatment the sample does not move along the column. In gas chromatography, there are also cases when a liquid sample is applied to the top of the column but its elution starts only after a given period. This term is useful in such cases.

3.7.07. Adjusted retention volume (time) (V'_R, t'_R) The total elution volume (time) minus the hold-up volume (time). It corresponds to the distance AB in Figure 1a:

$$V'_R = V_R - V_M$$

$$t'_R = t_R - t_M = (V_R - V_M)/F_c = V'_R/F_c$$

3.7.08. Corrected retention volume (time) ($V_R{}^\circ$, $t_R{}^\circ$) The total retention volume (time) multiplied by the compression correction factor (j):

$$V_R{}^\circ = V_R \,.\, j$$

$$t_R{}^\circ = V_R \,.\, j/F_c = V_R{}^\circ/F_c$$

3.7.09. Net retention volume (time) (V_N, t_N) The adjustment retention volume (time) multiplied by the compression correction factor (j):

$$V_N = V'_R \,.\, j$$

$$t_N = V'_R \,.\, j/F_c = V_N/F_c$$

3.7.10. In liquid chromatography, the compression of the mobile phase is negligible and thus, the compression correction factor does not apply. For this reason, the total and corrected retention volumes (times) are identical ($V_R = V_R^\circ$; $t_R = t_N$) and so are the adjusted and net retention volumes (times) ($V'_R = V_N$; $t'_R = t_N$).

3.7.11. Specific retention volumes

3.7.11.1. The specific retention volume at column temperature (V_g^θ) The net retention volume per gram of stationary phase (stationary liquid, active solid or solvent-free gel (W_S):

$$V_g^\theta = V_N/W_S$$

3.7.11.2. Specific retention volume at 0°C (V_g) The value of V_g^θ corrected to 0°C:

$$V_g = V_g^\theta \frac{273.15\ \text{K}}{T_c} = \frac{V_N}{W_S}\frac{273.15\ \text{K}}{T_c}$$

where T_c is the column temperature (in kelvin).

3.7.12. Retention factor (k) The retention factor is a measure of the time the sample component resides in the stationary phase relative to the time it resides in the mobile phase: it expresses how much longer a sample component is retarded by the stationary phase than it would take to travel through the column with the velocity of the mobile phase. Mathematically, it is the ratio of the adjusted retention volume (time) and the hold-up volume (time):

$$k = V'_R/V_M = t'_R/t_M$$

If the distribution constant (see 3.9) is independent of sample component concentration, then the retention factor is also equal to the ratio of the amounts of a sample component in the stationary and mobile phases respectively, at equilibrium:

$$k = \frac{\text{amount of component in stationary phase}}{\text{amount of component in mobile phase}}$$

If the fraction of the sample component in the mobile phase is R (see 3.7.13), then the fraction in the stationary phase is $(1 - R)$; thus

$$k = (1 - R)/R$$

Note: In former nomenclatures and in the literature one may find the expressions *Partition Ratio*, *Capacity Ratio*, *Capacity Factor* or *Mass Distribution Ratio* to describe this term.
In the literature the symbol k' is often used for the retention factor, particularly in liquid chromatography. The original reason for this was to clearly distinguish it from the partition coefficient (distribution constant) for which the symbol K had been utilized. Since, however, the distribution constants are all identified with a subscript, there is no reason to add the prime sign to this symbol. It should be emphasized that all the recognized nomenclatures (IUPAC, BS, ASTM) have always clearly identified the capacity factor with the symbol k and not k'.

3.7.12.1. Logarithm of the retention factor This term is equivalent to the R_M value used in planar chromatography (see 3.8.05). The symbol κ is suggested to express log k:

$$\kappa = \log k = \log[(1 - R)/R].$$

3.7.13. Retardation factor (*R*) The fraction of the sample component in the mobile phase at equilibrium; it is related to the retention factor and other fundamental chromatography terms:

$$R = 1/(k + 1)$$

3.7.14. Relative retention values

3.7.14.1. Relative retention (r) The ratio of the adjusted or net retention volume (time) or retention factor of a component relative to that of a standard, obtained under identical conditions:

$$r = V'_{Ri}/V'_{R(st)} = V_{Ni}/V_{N(st)} = t'_{Ri}/t'_{R(st)} = k_i/k_{st}$$

Depending on the relative position of the peak corresponding to the standard compound in the chromatogram, the value of r may be smaller, larger or identical to unity.

3.7.14.2. Separation factor (α) The relative retention value calculated for two adjacent peaks ($V'_{R2} > V'_{R1}$):

$$\alpha = V'_{R2}/V'_{R1} = V_{N2}/V_{N1} = t'_{R2}/t'_{R1} = k_2/k_1$$

By definition, the value of the separation factor is always greater than unity.

The separation factor is also identical to the ratio of the corresponding distribution constants.

Note: The separation factor is sometimes also called the 'selectivity'. The use of this expression is discouraged.

3.7.14.3. Unadjusted relative retention (r_G or α_G) Relative retention calculated by using the total retention volumes (times) instead of the adjusted or net retention volumes (times):

$$r_G = V_{Ri}/V_{R(st)} = t_{Ri}/t_{R(st)} = \frac{k_i + 1}{k_{st} + 1}$$

Subscript G commemorates E. Glueckauf, who first used this expression.

3.7.14.4. Relative retention (r) and separation factor (α) values must always be measured under isothermal conditions. On the other hand, the unadjusted relative retention (r_G or α_G) values may also be obtained in

programmed-temperature or gradient-elution conditions. Under such conditions, the symbol RRT (for *Relative Retention Time*) has also been used to describe the unadjusted relative retention values.

Using the same stationary and mobile phases and temperature, the relative retention and separation factor values are reproducible between chromatographic systems. On the other hand, the unadjusted relative retention (and 'relative retention time') values are only reproducible within a single chromatographic system.

3.7.15. Retention index; Kováts (retention) index (*I*) The retention index of a sample component is a number, obtained by interpolation (usually logarithmic), relating the adjusted retention volume (time) or the retention factor of the sample component to the adjusted retention volumes (times) of two standards eluted before and after the peak of the sample component.

In the *Kováts Index* or *Kováts Retention Index* used in gas chromatography, *n*-alkanes serve as the standards and logarithmic interpolation is utilized:

$$I = 100\left[\frac{\log X_i - \log X_z}{\log X_{(z+1)} - \log X_z} + z\right]$$

where X refers to the adjusted retention volumes or times, z is the number of carbon atoms of the *n*-alkane eluting before, and $(z + 1)$ is the number of carbon atoms of the *n*-alkane eluting after the peak of interest:

$$V'_{Rz} < V'_{Ri} < V_{R(z+1)}$$

The *Kováts* (*Retention*) *Index* expresses the number of carbon atoms (multiplied by 100) of a hypothetical normal alkane which would have an adjusted retention volume (time) identical to that of the peak of interest when analyzed under identical conditions.

The *Kováts Retention Index* is always measured under isothermal conditions. In the case of *temperature-programmed gas chromatography* a similar value can be calculated utilizing direct numbers instead of their logarithm. Since both the numerator and denominator contain the difference of two values, here we can use the total retention volumes (times). Sometimes this value is called the *Linear Retention Index*:

$$I^{T} = 100\left[\frac{t^{T}_{Ri} - t^{T}_{Rz}}{t^{T}_{R(z+1)} - t^{T}_{Rz}} + z\right]$$

where t^{T}_{R} refers to the total retention times (chart distances) measured under the conditions of temperature programming. The value of I^{T} will usually differ from the value of I measured for the same compound under isothermal conditions, using the same two phases.

3.8. Retention Parameters in Planar Chromatography

3.8.01. Mobile-phase front The leading edge of the mobile phase as it traverses the planar media. In all forms of development except radial, the mobile phase front is essentially a straight line parallel to the mobile phase surface. It is also called the *Liquid Front* or *Solvent Front*.

3.8.02. Mobile-phase distance The distance travelled by the mobile phase travelling along the medium from the starting (application) front or line to the mobile phase front. It is the distance *a* in Figure 2.

3.8.03. Solute distance The distance travelled by the solute along the medium from the starting (application) point or line to the center of the solute spot. If the solute spot is not circular, an imaginary circle is used whose diameter is the smallest axis of the spot. It is the distance *b* in Figure 2.

3.8.04. Retardation Factor (R_F) Ratio of the distance travelled by the center of the spot to the distance simultaneously travelled by the mobile phase. Using the symbols of Figure 2:

$$R_F = b/a$$

By definition the R_F values are always less than unity. They are usually given to two decimal places. In order to simplify this presentation the hR_F *Values* may be used: they correspond to the R_F values multiplied by 100.

Ideally, the R_F values are identical to the R values (see 3.7.13).

3.8.05. R_M value A logarithmic function of the R_F value:

$$R_M = \log\frac{1 - R_F}{R_F} = \log\left[\frac{1}{R_F} - 1\right]$$

3.8.06. Relative retardation (R_{rel}) This term is equivalent to relative retention used in column chromatography: it is the ratio of the R_F value of a component to the R_F value of a standard (reference) substance. Since the mobile phase front is common for the two components, the R_{rel} value can be expressed directly as the ratio of the distances travelled by the spot of the compound of interest (b_i) and the reference substance (b_{st}) respectively:

$$R_{rel} = R_{F(i)}/R_{F(st)} = b_i/b_{st}$$

Note: In former nomenclatures the symbol R_s was used to express relative retardation in planar chromatography. Because of its identity with the symbol for peak resolution (see 3.10.01) the symbol R_{rel} is suggested for relative retardation in planar chromatography.

3.9. Distribution Constants

The distribution constant is the concentration of a component in or on the stationary phase divided by the concentration of the component in the mobile phase. Since in chromatography a component may be present in more than one form (e.g. associated and dissociated forms), the analytical condition used here refers to the total amount present without regard to the existence of various forms.

There terms are also called the *Distribution Coefficients*. However, the present term conforms more closely to the general usage in science.

The concentration in the mobile phase is always calculated per unit volume of the phase. Depending on the way the concentration in the stationary phase is expressed various forms of the distribution constants may exist.

3.9.01. Distribution Constant (K_c) In the general case, the concentration in the stationary phase is expressed *per unit volume of the phase*. This term is mainly applicable to partition chromatography with a liquid stationary phase but can also be used with a solid stationary phase:

$$K_c = \frac{W_{i(S)}/V_S}{W_{i(M)}/V_M}$$

where $W_{i(S)}$ and $W_{i(M)}$ are the amounts of component i in the stationary and mobile phases, while V_S and V_M are the volumes of the stationary and mobile phases, respectively.

The term *Distribution Constant* and the symbol K_c are recommended in preference to the term *Partition Coefficient* which has been in use in partition chromatography with a liquid stationary phase.

The value of K_c is related to the retention volume (V_R) of a sample component and the volumes of the stationary (V_S) and mobile phases (V_M) in the column:

$$V_R = V_M + K_c V_S$$

In gas chromatography both V_R and V_M have to be corrected for gas compressibility: therefore V_R°(see 3.7.08) is to be used for V_R, and $V_G = V_M^\circ$ (see 3.2.11.2) is to be used for V_M.

$$V_R^\circ = V_G + K_c V_S$$

3.9.02. Distribution constant (K_g) In the case of a solid stationary phase, the distribution constant may be expressed *per mass (weight) of the dry solid phase*:

$$K_g = \frac{W_{i(S)}/W_S}{W_{i(M)}/V_M}$$

where $W_{i(st)}$ and $W_{i(M)}$ are the amounts (masses) of the component i in the stationary and mobile phases, respectively, W_{st} is the mass (weight) of the dry stationary phase, and V_M is the volume of the mobile phase in the column.

3.9.03. Distribution constant (K_s) In the case of adsorption chromatography with a well characterized adsorbent of known surface area, the concentration in the stationary phase may be expressed *per unit surface area*:

$$K_s = \frac{W_{i(S)}/A_S}{W_{i(M)}/V_M}$$

where $W_{i(S)}$ and $W_{i(M)}$ are the amounts (masses) of the component i in the stationary and mobile phases, respectively, A_S is the surface area of the stationary phase, and V_M is the volume of the mobile phase in the column.

Note: The symbols used in 3.9.01 through 3.9.03 are generalized.

3.10. Terms Expressing the Efficiency of Separation

3.10.01. Peak resolution (R_s) The separation of two peaks in terms of their average peak width at base ($t_{R2} > t_{R1}$):

$$R_s = \frac{(t_{R2} - t_{R1})}{(w_{b1} + w_{b2})/2} = \frac{2(t_{R2} - t_{R1})}{w_{b1} + w_{b2}}$$

In the case of two adjacent peaks it may be assumed that $w_{b1} \approx w_{b2}$, and thus, the width of the second peak may be substituted for the average value:

$$R_s \approx (t_{R2} - t_{R1})/w_{b2}$$

3.10.02. Separation number (SN) This expresses the number of peaks which can be resolved in a given part of the chromatogram between the peaks of two consecutive *n*-alkanes with z and $(z + 1)$ carbon atoms in their molecules:

$$\mathrm{SN} = \frac{t_{R(z+1)} - t_{Rz}}{w_{hz} + w_{h(z+1)}} - 1$$

In the German literature the symbol TZ (*Trennzahl*) is commonly used to express the separation number.

As the separation number depends on the *n*-alkanes used for the calculation, they always must be specified with any given SN value.

3.10.03. Plate number (N) A number indicative of column performance, calculated from the following equations which depend on the selection of the peak width expression (see 3.3.07):

$$N = (V'_R/\sigma)^2 = (t_R/\sigma)^2$$

$$N = 16(V'_R/w_b)^2 = 16(t_R/w_b)^2$$

$$N = 5.545(V'_R/w_h)^2 = 5.545(t_R/w_h)^2$$

The value of 5.545 stands for 8 ln 2 (see 3.3.07.4). These expressions assume a Gaussian (symmetrical) peak.

In these expressions the units for the quantities inside the brackets must be consistent so that their ratio is dimensionless: i.e., if the numerator is a volume, then peak width must also be expressed in terms of volume.

Note: In former nomenclatures the expressions 'Number of Theoretical Plates' or 'Theoretical Plate Number' were used for the same term. For simplification, the present name is suggested.

3.10.04. Effective plate number (N_{eff}) A number indicative of column performance calculated by using the adjusted retention volume (time) instead of the total retention volume (time). It is also called the *Number of Effective Plates*:

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

$$N_{eff} = 16(V'_R/w_b)^2 = 16(t'_R/w_b)^2$$

$$N_{eff} = 5.545(V'_R/w_h)^2 = 5.545(t'_R/w_h)^2$$

The plate number and effective plate number are related to each other:

$$N = N_{eff}\left[\frac{k+1}{k}\right]^2$$

Where k is the retention factor (see 3.7.12).

Notes: In the former literature the expression 'number of effective theoretical plates' had been used to express this term. This is incorrect since the plate number is either theoretical or effective, but cannot be both.
In former nomenclatures the respective symbols n and N have been used for the plate number and the effective plate number. However, there was often a confusion in the proper selection of lower case and capital letters; therefore, the present usage, characterizing the effective plate number by a subscript, is suggested.

3.10.05. Plate height (H) The column length (L) divided by the plate number:

$$H = L/N$$

It is also called the *Height Equivalent to One Theoretical Plate* (HETP).

3.10.06. Effective plate height (H_{eff}) The column length divided by the effective plate number:

$$H_{eff} = L/N_{eff}$$

It is also called the *Height Equivalent to One Effective Plate*.

Notes: In the former literature the expression 'height equivalent to one effective theoretical plate' had been used to express this term. This is incorrect, since the plate height is either theoretical or effective (see 3.10.04), but cannot be both.
In former nomenclatures the respective symbols h and H have been used for the plate height and the effective plate height, respectively. However, there was often a confusion in the proper selection of lower case and capital letters and also due to the fact that h (lower case letter) is also used to express the reduced plate height (see 3.10.07). The present usage is suggested in order to avoid any confusion.

3.10.07. Reduced plate height (h) A term used in liquid chromatography. It is the ratio of the plate height to the average particle diameter:

$$h = H/d_p$$

For open-tubular columns:

$$h = H/d_c$$

4. Terms Related to Detection

4.1. Classification of Detectors

4.1.01. Classification according to the form of response

4.1.01.1. Differential detectors These measure the instantaneous difference in the composition of the column effluent.

4.1.01.2. Integral detectors These measure the accumulated quantity of sample component(s) reaching the detector.

4.1.02. Classification according to the basis of response

4.1.02.1. Concentration-sensitive detector A device the response of which is proportional to the concentration of a sample component in the eluent.

4.1.02.2. Mass-flow sensitive detector A device the response of which is proportional to the amount of sample component reaching the detector in unit time.

4.1.03. Classification according to Detector selectivity

4.1.03.1. Universal detector A detector which responds to every component in the column effluent except the mobile phase.

4.1.03.2. Selective detector A detector which responds to a related group of sample components in the column effluent.

4.1.03.3. Specific detector A detector which responds to a single sample component or to a limited number of components having similar chemical characteristics.

4.2. Detector Response

4.2.01. Detector sensitivity (*S*) The signal output per unit concentration or unit mass of a substance in the mobile phase entering the detector.

4.2.01.1. In the calculation of detector sensitivity the signal output of the detector is given as peak area in mV.min, A.s or AU.min (AU = absorbance unit). These values are obtained from the *integrated* peak area converted to the units specified.

Alternately, the peak area can also be obtained by multiplying the peak height at maximum (in mV, A or AU) by the peak-width at half height (in time units). The peak area calculated in this way will be 6% less than the true integrated peak area, assuming that peak is Gaussian.

4.2.01.2. In the case of *concentration-sensitive detectors*, sensitivity is calculated per unit concentration in the mobile phase:

$$S = A_i F_c / W_i = E / C_i$$

where A_i is the integrated peak area (in mV.min or AU.min), E is the peak height (in mV or AU), C_i is the concentration of the particular substance in the mobile phase at the detector (in $g.cm^{-3}$), F_c is the mobile phase flow rate corrected to column temperature (in $cm^3.min^{-1}$) and W_i is the mass (amount) of the substance present (in mg). The dimensions of detector sensitivity are $mV.cm^3.mg^{-1}$ or $AU.cm^3.mg^{-1}$.

4.2.01.3. In the case of thermal-conductivity detectors, this sensitivity value is also called the *Dimbat-Porter-Stross Sensitivity* of the detector.

In the case of *mass-flow sensitive detectors*, sensitivity is calculated per unit mass of the test substance in the mobile phase entering the detector:

$$S = A_i / W_i = E_i / M_i$$

where A_i is the integrated peak area (in A.s), E_i is the peak height (in A), M_i is the mass rate of the test substance entering the detector (in $g.s^{-1}$), and W_i is the mass (amount) of test substance present (in g). The dimension of detector sensitivity is $A.s.g^{-1}$ or $C.g^{-1}$.

4.2.02. Relative detector response factor (*f*) The relative detector response factor expresses the sensitivity of a detector relative to a standard substance. It can be expressed on an equal mole, equal volume or equal mass (weight) basis:

$$f_i = (A_i/A_{st})f_{st}$$

where A refers to the peak area of the compound of interest (subscript i) and standard (subscript st) respectively, and f_{st} is the response factor of the standard compound. Usually, an arbitrary value (e.g. 1 or 100) is assigned to f_{st}. Expressing the relative molar responses and using *n*-alkanes as the standards, the assigned value of f_{st} is usually the number of carbon atoms of the *n*-alkanes multiplied by 100 (e.g. 600 for *n*-hexane).

If the relative detector response factor is expressed on an equal mass (weight) basis, the determined sensitivity values can be substituted for the peak area.

4.3. Noise and Drift

4.3.01. Noise (*N*) (see Figure 4) The amplitude expressed in volts, amperes, or absorbance units of the envelope of the baseline which includes all random variations of the detector signal the frequency of which is in the order of one or more cycles per minute. In the case of photometric detectors the amplitude may be expressed in absorbance units per unit cell length.

4.3.02. Drift (see Figure 4) The average slope of the noise envelope, expressed in volts, amperes, or absorbance units per hour. It may be actually measured for 0.5 hour and extrapolated to one hour.

4.4. Minimum Detectability

The concentration or mass flow of a sample components in the mobile phase gives a detector signal equal to twice the noise level. It can be calculated from the measured sensitivity (S) and noise (N):

$$D = 2N/S$$

where D is the minimum detectability, expressed either as concentration or mass-flow of the substance of interest in the mobile phase at the detector. Both sensitivity and minimum detectability must be determined for the same substance.

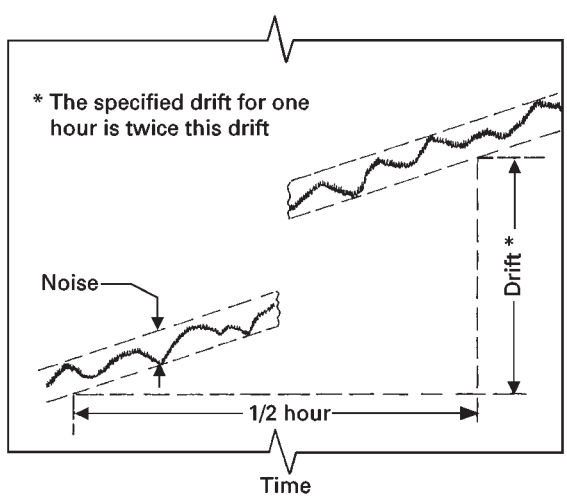

Figure 4 Measurement of the noise and drift of a chromatographic detector.

4.5. Linear and Dynamic Ranges

4.5.01. Linear range The linear range of a chromatographic detector represents the range of concentrations or mass flows of a substance in the mobile phase at the detector over which the sensitivity of the detector is constant within a specified variation, usually ± 5 percent.

4.5.01.1. The best way to present detector linear range is the *Linearity Plot* (*see* **Figure 5**) plotting detector sensitivity against amount injected, concentration or mass flow-rate. Here, the upper limit of linearity can be graphically established as the amount, concentration, or mass flow-rate) at which the deviation exceeds the specified value ($\pm x\%$ window around the plot). The lower limit of linearity is always the minimum detectable amount determined separately for the same compound.

4.5.01.2. Alternatively, the linear range of a detector may be presented as the plot of peak area (height) against concentration or mass flow-rate of the test substance in the column effluent at the detector (see **Figure 6**). This plot may be either linear or log/log. The upper limit of linearity is that concentration (mass flow-rate) at which the deviation from an ideal linearity plot is greater than the specified percentage deviation ($\pm x\%$ window).

4.5.01.3. Numerically, the linear range can be expressed as the ratio of the upper limit of linearity obtained from the linearity plot and the minimum detectability, both measured for the same substance.

4.5.01.4. When presenting the linear range of a detector, either as a plot as a numerical value, the test substance, the minimum detectability, and the specified deviation must be stated.

4.5.02.

4.5.02.1. Dynamic range The dynamic range of detector is that range of concentration or mass flow-rates of a substance over which an incremental change in concentration or mass flow-rate produced an incremental change in detector signal. Figure 6 Presents a plot used for the determination of the dynamic range of a detector.

4.5.02.2. The lower limit of the dynamic range is the minimum detectability. The upper limit is the highest concentration at which a further increase in concentration (mass flow-rate) will still give an observable

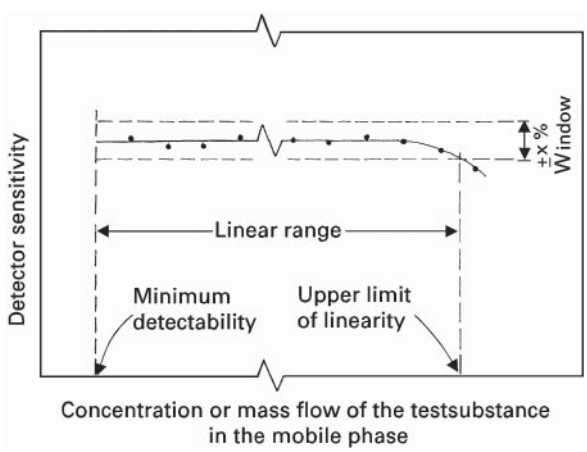

Figure 5 Linearity plot of a chromatographic detector. The scale of the ordinate is linear: the scale of the abscissa may be either linear or logarithmic.

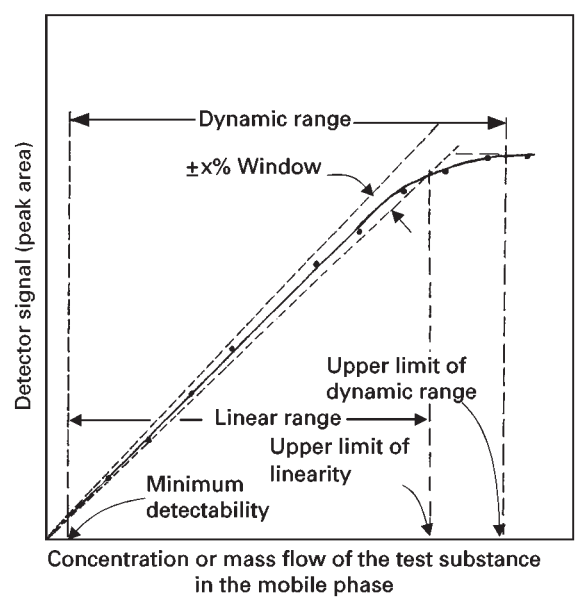

Figure 6 Determination of the linear and dynamic ranges of a chromatographic detector. Such a plot is usually in a log-log scale.

increase in detector signal, and the dynamic range is the ratio of the upper and lower limits. The dynamic range is greater than the linear range.

4.5.02.3. Numerically the dynamic range can be expressed as the ratio of the upper limit of the dynamic range obtained from the plot and the minimum detectability, both measured for the same substance.

4.5.02.4. When expressing the dynamic range of a detector, the test substance and the minimum detectability must be stated.

Table 1 Index of terms

Compound terms are generally listed at two places: under the main term and also under the full name. Exception was made when both the main term and its adjective start with same letter. For example, ion-exchange chromatography is listed as 'ion-exchange chromatography' and 'chromatography, ion-exchange'; on the other hand, column chromatography is listed only as 'chromatography, column.' The numbers refer to the relevant sections. Terms specifically used in planar chromatography (PC), ion-exchange chromatography (IEC) and exclusion chromatography (EC) are indicated by the corresponding acronyms.

A	
Active solid	3.1.01
Adjusted retention time	3.7.07; (EC) 6.2.03
Adjusted retention volume	3.7.07; (EC) 6.2.03
Adsorption chromatography	1.5.01
Affinity chromatography	1.5.05
Ambient pressure	3.6.02.2
Ambient temperature	3.5.01
Anion exchange	(IEC) 5.1.10
Anion-exchange membrane	(IEC) 5.3.04
Anion exchanger	(IEC) 5.3.03
- basic form	5.3.03.1
Anion-exchange resin	(IEC) 5.3.03
Anticircular development	(PC) 2.2.02.6
Application point (line)	(PC) 3.3.03
Ascending elution (development)	(PC) 2.2.02.2
B	
Band	3.3.05
Baseline	3.3.05
Bed density	(IEC) 5.6.03
Bed volume	3.2.06
Bed volume capacity	(IEC) 5.4.03
Bifunctional ion exchanger	(IEC) 5.3.01.3
Binder	(PC) 3.1.04
Bonded phase	1.1.05.1
Break-through capacity	(IEC) 5.4.05
Bypass injector	2.1.02.2
C	
Capacity	(IEC)
- bed-volume	5.4.03
- break-through	5.4.05
- practical specific	5.4.04
- theoretical specific	5.4.01
- volume	5.4.02
Capacity factor	3.7.12; (EC) 6.2.05
Capacity ratio	3.7.12
Capillary column	3.2.04
- open-tubular	3.2.03
- packed	3.2.04
Carrier gas	1.1.06
- velocities	3.6.05.2
Cation exchange	(IEC) 5.1.09
Cation-exchange membrane	(IEC) 5.3.04
Cation exchanger	(IEC) 5.3.02
- acid form	5.3.02.1
Chamber saturation	(PC) 2.2.02.7
Chromatogram	1.1.02
- differential	3.3.01
- integral	3.3.02
Chromatograph	1.1.03; 1.1.04
Chromatographic bed	1.1.05
Chromatography	1.1.01
- adsorption	1.5.01
- affinity	1.5.05
- column	1.3.01
- displacement	1.2.02
- elution	1.2.03
- exclusion	1.5.04
- frontal	1.2.01
- gas	1.1.06; 1.4.02
- gas-liquid	1.4.01
- gas-solid	1.4.01
- gel-permeation	1.5.04
- ion	1.5.03
- ion-exchange	1.5.03
- ion-exclusion	1.5.04
- isothermal	1.6.07
- liquid	1.1.06; 1.4.03
- liquid-liquid	1.4.01
- liquid-solid	1.4.01
- normal-phase	1.6.02
- open-bed	1.3.02
- paper	1.3.02
- partition	1.5.02
- planar	1.3.02
- programmed-flow	1.6.09
- programmed-temperature	1.6.08
- pyrolysis-gas	1.6.11.1
- reaction	1.6.11
- reversed-phase	1.6.01
- supercritical-fluid	1.4.04
- thin-layer	1.3.02
- two-dimensional	1.6.06
Circular elution (development)	(PC) 2.2.02.5
Co-ions	(IEC) 5.1.08
Column	3.2
- capillary	3.2.04
- cross-sectional area	3.2.10
- diameter	3.2.07
- interparticle porosity	3.2.12
- interparticle volume	3.2.11; (EC) 6.1.01
- interstitial fraction	3.2.12

Table 1 *Continued*

Column	3.2
- interstitial volume	3.2.11; (EC) 6.1.01
- intraparticle volume	(EC) 6.1.02
- intrastitial volume	(EC) 6.1.02
- length	3.2.02
- open-tubular	3.2.03
- packed	3.2.02
- radius	3.2.08
- temperature	2.1.03
- void volume	3.2.11
- volume	3.2.05
Column oven	2.1.03
Compression (Compressibility) correction factor	3.6.03
Concentration-sensitive detectors	4.1.02.1
Corrected gas hold-up volume (time)	3.7.04
Corrected retention volume (time)	3.7.08
Corrected selectivity coefficient	(IEC) 5.5.03
Counter-ions	(IEC) 5.1.02
D	
Dead volume	3.2.13.1
Densitometer	(PC) 2.2.04
Derivatisation	
- post-column	1.6.11.2
- pre-column	1.6.11.2
Descending elution (development)	(PC) 2.2.02.4
Detector	2.1.05
- concentration-sensitive	4.1.02.1
- differential	4.1.01.1
- dynamic range	4.5.02
- integral	4.1.01.2
- linearity plot	4.5.01.2
- linear range	4.5.01
- mass-sensitive	4.1.02.2
- minimum detectability	4.4
- relative response factor	4.2.02
- selective	4.1.03.2
- sensitivity	4.2.01
- specific	4.1.03.3
- universal	4.1.03.1
Develop	(PC) 1.1.07
Developing chamber	(PC) 2.2.02
Development (PC)	
- anticircular	2.2.02.6
- ascending	2.2.02.2
- circular	2.2.05.5
- descending	2.2.02.4
- horizontal	2.2.02.3
- radial	2.2.02.5
- saturated	2.2.02.7
- unsaturated	2.2.02.8
Differential chromatogram	3.3.01
Diffusion	3.4
Diffusion coefficient	
- in ion exchanger	(IEC) 5.5.01
- in mobile phase	3.4.03
- in stationary phase	3.4.02
Diffusion velocity	3.4.04
Dimbat-Porter-Stross sensitivity	4.2.01.2
Direct injector	2.1.02.1
Displacement chromatography	1.2.02
Displacer	1.2.02
Distribution constants	3.9; (IEC) 5.6; (EC) 6.2.06
Drift	4.3.02
Dynamic range (of detector)	4.5.02
E	
Effective plate height	3.10.06; (EC) 6.3.04
Effective plate number	3.10.04; (EC) 6.3.04
Effluent	1.1.08
Electron exchanger	(IEC) 5.3.01.7
Elute	1.1.07
Eluent	1.1.06
Elution	
- anticircular	2.2.02.6
- ascending	2.2.02.2
- chamber	2.2.02
- circular	2.2.02.5
- descending	2.2.02.4
- horizontal	2.2.02.3
- radial	2.2.02.5
- unsaturated	2.2.02.8
Elution chromatography	1.2.03
Equilibration	(PC) 2.2.02.9
Exclusion chromatography	1.5.04
External solution	(IEC) 5.2.02
External standard	3.3.12
Extracolumn volume	3.2.13
F	
Film thickness (of the liquid phase)	3.2.14
Final isothermal period	3.5.04.5
Final isothermal temperature	3.5.04.5
Final temperature	3.5.04.4
Fixed ions	(IEC) 5.1.03
Flash vaporizer	2.1.02.4
Flow programming	1.6.09
Flow-rates	3.6.04
Flow-resistance parameter	3.2.19
Fraction collector	2.1.04
Frontal chromatography	1.2.01
Fronting	3.3.09
G	
Gas chromatography	1.4.02
Gas hold-up time	3.7.03
Gas hold-up volume	3.7.03
- corrected	3.7.04
Gas-liquid chromatography	1.4.01
Gas phase	1.1.05
Gas sampling valve	2.1.02.7
Gas-solid chromatography	1.4.01
Gel-permeation chromatography	1.5.04
Gradient elution	1.6.04
Gradient layer	3.1.05
H	
Height equivalent to one effective plate	3.10.06; (EC) 6.3.04
Height equivalent to one theoretical plate	3.10.05; (EC) 6.3.03
Heterogeneous ion-exchange membrane	(IEC) 5.3.04
High-performance liquid chromatography	1.4.03
- - thin-layer chromatography	1.4.03

Table 1 *Continued*

Hold-up time	3.7.03
Hold-up volume	3.7.03
- corrected	3.7.04
Homogeneous ion-exchange membrane	(IEC) 5.3.04
Horizontal elution (development)	(PC) 2.2.02.3
I	
Immobilized phase	1.1.05.2
Impregnation	(PC) 3.1.06
Initial isothermal period	3.5.04.1
Initial isothermal temperature	3.5.04.1
Initial temperature	3.5.04.1
Injection temperature	3.5.02
Injector	2.1.02
- bypass	2.1.02.2
- direct	2.1.02.1
- flash vaporizer	2.1.02.4
- on-column	2.1.02.3
- programmed-temperature vaporizer	2.1.02.6
- split	2.1.02.5
- valve	2.1.02.2
Inlet pressure	3.6.02.1
Integral chromatogram	3.3.02
Integral detector	4.1.01.2
Internal standard	3.3.1
Interparticle porosity	3.2.1.2
Interparticle volume	3.2.11; (EC) 5.1.01
Interstitial fraction	3.2.12
Interstitial volume	3.2.11; (EC) 6.1.01
Intraparticle volume	(EC) 6.1.02
Intrastitial volume	(EC) 6.1.02
Ion chromatography	1.5.03
Ion-exchange chromatography	1.5.03
Ion exchange	(IEC) 5.1.01
- isotherm	5.1.04
Ion-exchange membrane	(IEC) 5.3.04
Ion exchanger	(IEC) 5.3.01
- anion	5.3.03
- bifunctional	5.3.01.3
- cation	5.3.02
- macroporous	5.3.01.5
- monofunctional	5.3.01.2
- polyfunctional	5.3.01.4
- redox	5.3.01.8
- salt form	5.3.01.6
Ion-exclusion chromatography	1.5.04
Ionogenic group	5.1.07
Isocratic analysis	1.6.03
Isothermal chromatography	1.6.07
Isothermal period	
- final	3.5.04.5
- initial	3.5.04.1
- mid-analysis	3.5.04.3
K	
Kováts Retention Index	3.7.15
L	
Linearity plot (of detector)	4.5.01.2
Linear range (of detector)	4.5.01
Linear retention index	3.7.15
Liquid chromatography	1.4.03
Liquid-liquid chromatography	1.4.01
Liquid phase	1.1.05
- film thickness	3.2.14
- loading	3.1.10
- mass (weight)	3.2.16
- volume	3.2.15
Liquid-solid chromatography	1.4.01
Liquid stationary phase	1.1.05
M	
Macroporous ion exchanger	5.3.01.5
Marker	3.3.13
Mass distribution ratio	3.7.12
Mass-flow sensitive detectors	4.1.02.2
Mid-analysis isothermal period	3.5.04.3
Mid-analysis isothermal temperature	3.5.04.3
Minimum detectability	4.4
Mobile phase	1.1.06
- compression correction factor	3.6.03
- distance	(PC) 3.8.02
- flow-rates	3.6.04
- front	(PC) 3.8.01
- reduced	3.6.05.3
- velocities	3.6.05.1; 3.6.05.2
- viscosity	3.6.01
Modified active solid	3.1.02
Monofunctional ion exchanger	5.3.01.2
Multi-dimensional chromatography	1.6.06
Multiple programming	3.5.04.2
N	
Net retention volume (time)	3.7.09
Noise	4.3.01
Normal-phase chromatography	1.6.02
Number of effective plates	3.10.04; (EC) 6.3.04
Number of theoretical plates	3.10.03; (EC) 6.3.03
O	
On-column injector	2.1.02.3
Open-bed chromatography	1.3.02
Open-tubular column	3.2.03
- porous-layer	3.2.03.2
- support-coated	3.2.03.3
- wall-coated	3.2.04.1
Outlet pressure	3.6.02.2
Oven	2.1.03
P	
Packing	3.1.07
- pellicular	3.1.07.2
- totally porous	3.1.07.1
Packed capillary column	3.2.04
Packed column	3.2.02
Paper chromatography	(PC) 1.3.02
Particle diameter	3.1.08
Partition chromatography	1.5.02
Partition coefficient	3.9.01
Partition ratio	3.7.12
Peak	3.3.06
- area	3.3.06.2
- base	3.3.06.1
- fronting	3.3.09

Table 1 *Continued*

Peak	3.3.06
- height	3.3.06.4
- maximum	3.3.06.3
- resolution	3.10.01; (EC) 6.3.01
- standard deviation	3.3.06.5
- tailing	3.3.08
- variance	3.3.06.6
- widths	3.3.07
Peak elution volume (time)	3.7.06
Pellicular packing	3.1.07.2
Permeability, specific	3.2.18
Perm-selectivity	(IEC) 5.3.04.1
Phase	
- immobilized	1.6.02
- mobile	1.1.06; 3.6
- stationary	1.1.05
Phase ratio	3.2.17
Planar chromatography	1.3.02
Plate height	3.10.05; (EC) 6.3.03
- effective	3.10.06; (EC) 6.3.04
- reduced	3.10.07; (EC) 6.3.05
- theoretical	3.10.05; (EC) 6.3.03
Plate number	3.10.03; (EC) 6.3.03
- effective	3.10.04; (EC) 6.3.04
- theoretical	3.10.03; (EC) 6.3.03
Pneumatic pump	2.1.01.3
Pore radius	3.1.09
Porous-layer open-tubular column	3.2.03.2
Post-column derivatization	1.6.11.2
Practical specific capacity	(IEC) 5.4.04
Pre-column derivatization	1.6.11.2
Pressure	3.6.02
- ambient	3.6.02.2
- drop	3.6.02.3
- inlet	3.6.02.1
- outlet	3.6.02.2
- programming	3.6.11
- relative	3.6.02.4
Programmed-flow chromatography	1.6.09
Programmed pressure chromatography	1.6.10
Programmed-temperature chromatography	1.6.08
Programmed-temperature vaporizer	2.1.02.6
Program rate	3.5.04.2
Pumps	2.1.01
- pneumatic	2.1.01.3
- reciprocating	2.1.01.2
- syringe	2.1.01.1
Pyrolysis-gas chromatography	1.6.11.1
R	
Radial elution (development)	(PC) 2.2.02.5
Reaction chromatography	1.6.11
Reciprocating pump	2.1.01.2
Redox ion exchanger	(IEC) 5.3.01.8
Redox polymers	(IEC) 5.3.01.7
Reduced plate height	3.10.07; (EC) 6.3.05
Reduced velocity (of mobile phase)	3.6.05.3
Relative pressure	3.6.02.4
Relative response factor (of detector)	4.2.02
Relative retardation	(PC) 3.8.06
Relative retention	3.7.14
- unadjusted	3.7.14.3
Relative retention time	3.7.14.4
R_M value	(PC) 3.8.05
Resin matrix	(IEC) 5.3.01.1
Resolution	3.10.01; (EC) 6.3.01
Retardation factor	3.7.13; (PC) 3.8.04
Retention factor	3.7.12; (EC) 6.2.05
- logarithm	3.7.12.1
Retention index	3.7.15
Retention parameters	3.7; (PC) 3.8
Retention temperature	3.5.04.6
Retention times	
- adjusted	3.7.07; (EC) 6.2.03
- corrected	3.7.08
- net	3.7.09
- peak elution	3.7.06
- total	3.7.05; (EC) 6.2.02
- total mobile-phase	6.2.04
- unretained compound	3.7.03; (EC) 6.2.02
Retention volumes	
- adjusted	3.7.07; (EC) 6.2.03
- corrected	3.7.08
- net	3.7.09
- peak elution	3.7.06
- specific	3.7.11
- total	3.7.05; (EC) 6.2.02
- total mobile phase	(EC) 6.2.04
- unretained compound	3.7.03; (EC) 6.2.01
Reversed-phase chromatography	1.6.01
S	
Salt form of ion exchanger	(IEC) 5.3.01.6
Sample	1.1.09
- component	1.1.10
- injector	2.1.02
Sampling valve	2.1.02.2
Selective detectors	4.1.03.2
Selectivity coefficient	(IEC) 5.5.02
- corrected	5.5.03
Sensitivity of detectors	4.2.01
- Dimbat-Porter-Stross	4.2.01.2
Separation factor	3.7.14.2; (IEC) 5.5.04
Separation number	3.10.02
Separation temperature	2.1.03
Solid stationary phase	1.1.05
Solid support	3.1.03
Solute	1.1.11
Solute distance	(PC) 3.8.03
Solvent	1.1.12; (IEC) 5.2.01
Sorption	(IEC) 5.1.05
- isotherm	(IEC) 5.1.06
Specific capacity (IEC)	
- practical	5.4.04
- theoretical	5.4.01
Specific detectors	4.1.03.3
Specific permeability	3.2.18
Specific resolution	(EC) 6.3.02
Specific retention volume	3.7.11
Split injection	2.1.02.5
Spot	(PC) 3.3.04
- diameter	3.3.04.1
Spotting device	(PC) 2.2.01

Table 1 *Continued*

Standard deviation	3.3.06.5	Theoretical plate number	3.10.03; (EC) 6.3.03
Starting point	(PC) 3.3.03	Theoretical specific capacity	(IEC) 5.4.01
Stationary mobile-phase volume	(EC) 6.1.03	Thin-layer chromatography	1.3.02
Stationary phase	1.1.05	Total mobile-phase time	(EC) 6.2.04
- volume	3.2.15	Total mobile-phase volume	(EC) 6.1.03; 6.2.04
- mass (weight)	3.2.16	Totally porous packing	3.1.07.1
Step	3.3.10	Trennzahl	3.10.02
- height	3.3.10.1	Two-dimensional chromatography	1.6.06
Stepwise elution	1.6.05		
Supercritical-fluid chromatography	1.4.04	***U***	
Support	3.1.03	Unadjusted relative retention	3.7.14.3
Support-coated open-tubular column	3.2.03.2	Universal detectors	4.1.03.1
Syringe pump	2.1.01.1	Unsaturated elution (development)	(PC) 2.2.02.8
T		***V***	
Tailing	3.3.08	Valve injector	2.1.02.2
Temperature		Variance	3.3.06.6
- ambient	3.5.01	Velocities (of mobile phase)	3.6.05.1
- column	2.1.03; 3.5.03	Viscosity (of mobile phase)	3.6.01
- detector	3.5.05	Visualization chamber	(PC) 2.2.03
- final	3.5.04.4	Void volume	3.2.11
- final isothermal	3.5.04.5	Volume capacity	(IEC) 5.4.02
- initial	3.5.04.1	Volume swelling ratio	(IEC) 5.3.06
- initial isothermal	3.5.04.1		
- injection	3.5.02	***W***	
- mid-analysis isothermal	3.5.04.3	Wall-coated open tubular column	3.2.03.1
- program rate	3.5.04.2	Weight-swelling ratio in solvent	(IEC) 5.3.05
- retention	3.5.04.6		
- separation	2.1.03; 3.5.03	***Z***	
Theoretical plate height	3.10.05; (EC) 6.3.03	Zone	1.1.13

Table 2 List of symbols

The numbers in parentheses refer to the relevant sections. Symbols used specifically in planar chromatography (PC), ion-exchange chromatography (IEC) or exclusion-chromatography (EC) are indicated by the corresponding acronyms.

a	Mobile phase-distance in PC (3.8.02)
A	Peak area (4.2.01)
A_c	Cross-sectional area of a column (3.2.10)
A_S	Surface area of stationary phase in column (3.9.03)
b	Solute distance in PC (3.8.03)
B_o	Specific permeability (3.2.18)
C_i	Concentration of a test substance in the mobile phase at the detector (4.2.01.2)
d_c	Column inside diameter (3.2.07)
d_f	Thickness of liquid phase film (3.2.14)
d_p	Particle diameter (3.1.08)
D	Minimum detectability of a detector (4.4)
D	Diffusion coefficient in general (3.4)
D_{ex}	Diffusion coefficient in an ion exchanger (5.5.01)
D_G	Diffusion coefficient in the gas phase (3.4.03)
D_L	Diffusion coefficient in the liquid stationary phase (3.4.02)
D_M	Diffusion coefficient in the mobile phase (3.4.03)
D_S	Diffusion coefficient in the stationary phase (3.4.02)
E	Peak height (4.2.01.2)
f	Relative detector response factor (4.2.02)
F	Mobile-phase flow-rate, measured at column outlet under ambient conditions with a wet flowmeter (3.6.04.1)
F_a	Mobile-phase flow-rate at ambient temperature (3.6.04.1)
F_c	Mobile-phase flow-rate, corrected to column temperature (3.6.04.2)
h	Reduced plate height (3.10.07)
hR_F	RF $\times$ 100 (3.8.04)
H	Plate height (height equivalent to one theoretical plate) (3.10.05)
H_{eff}	Effective plate height (height equivalent to one effective plate) (3.10.06; in EC: 6.3.04)

Table 2 *Continued*

I	Retention index; Kováts (retention) index (3.7.15)
I^T	Retention index obtained in programmed temperature analysis; Linear retention index (3.7.15)
j	Mobile phase compression (compressibility) correction factor (3.6.03)
k	Retention factor (capacity factor) (3.7.12)
k_e	Retention factor (capacity factor) in EC (6.2.05)
$k_{A,B}$	Selectivity coefficient in IEC (5.5.02)
$k^a_{A,B}$	Corrected selectivity coefficient (IEC) (5.5.03)
K	Distribution constants in general (3.9)
K_c	Distribution constant in which the concentration in the stationary phase is expressed as mass of substance per volume of the phase (3.9.01). In IEC, it refers to unit volume of the swollen ion exchanger (5.6.01)
K_g	Distribution constant in which the concentration in the stationary phase is expressed as mass of substance per mass of the solid phase (3.9.02). In IEC, it refers to unit mass of the dry ion exchanger (5.6.02)
K_s	Distribution constant in which the concentration in the stationary phase is expressed as mass of substance per surface area of the solid phase (3.9.03)
K_v	Distribution constant used in IEC, in which the concentration in the stationary phase is expressed as volume of substance per volume of the dry ion exchanger (5.6.03)
K_o	Distribution constant in EC (6.2.06)
L	Column length (3.2.09)
M	in EC: molecular mass (6.3.01 & 6.3.02)
M_i	Mass rate of the test substance entering the detector (4.2.01.3)
N	Noise of a detector (4.3.01)
N	Plate number (number of theoretical plates) (3.10.03)
N_{eff}	Effective plate number (number of effective plates) (3.10.04; in EC: 6.3.04)
p	Pressure in general (3.6.02)
p_a	Ambient pressure (3.6.02.2)
p_i	Inlet pressure (3.6.02.1)
p_o	Outlet pressure (3.6.02.2)
p_w	Partial pressure of water at ambient temperature (3.6.04.1)
Δp	Pressure drop (3.6.02.3)
P	Relative pressure (3.6.02.4)
Q_A	Practical specific capacity of an ion exchanger (5.4.04)
Q_B	Break-through capacity of an ion-exchange bed (5.4.05)
Q_V	Volume capacity of an ion exchanger (5.4.02)
r	Relative retention (3.7.14.1)
r_c	Inside column radius (3.2.08)
r_G	Unadjusted relative retention (3.7.14.3)
r_p	Pore radius (3.1.09)
R	Retardation factor in column chromatography; fraction of a sample component in the mobile phase (3.7.12 & 3.7.13)
$(R-1)$	Fraction of a sample component in the stationary phase in column chromatography (3.7.12)
R_F	Retardation factor in PC (3.8.04)
R_M	Logarithmic function of R_F (PC) (3.8.05)
R_{rel}	Relative retardation in PC (3.8.06)
R_s	Peak resolution (3.10.01)
R_{sp}	Specific resolution in EC (6.3.02)
$R_{1/2}$	Peak resolution in EC (6.3.02)
S	Detector sensitivity (4.2.01)
SN	Separation number
t	Time in general
t_i	Retention time corresponding to the interparticle volume (V_i) of the column (EC) (6.1.02)
t_t	Retention time corresponding to the total mobile phase volume (V_t) in the column (EC) (6.2.04)
t_o	Retention time of an unretained compound in EC (6.2.01)
t_M	Mobile-phase hold-up time; except in EC (see 6.1.01) it is also equal to the retention time of an unretained compound (3.7.03)
t_N	Net retention time (3.7.09)
t_R	Total retention time (3.7.05; in EC: 6.2.02)
t^T_R	Total retention time in temperature-programmed analysis (3.7.05 & 3.7.15)
$\overline{t_R}$	Peak elution time (3.7.06)
t'_R	Adjusted retention time (3.7.07; in EC: 6.2.03)
t^o_R	Corrected retention time (3.7.08)
T	Temperature in general (always in kelvin) (3.5)
T_a	Ambient temperature (3.5.01)
T_c	Column temperature (3.5.03)
TZ	Trennzahl number (separation number) (3.10.02)
u	Mobile-phase velocity (3.6.05.1)

Table 2 *Continued*

$\bar{u}$	Average linear carrier gas velocity (3.6.05.2)
u_D	Diffusion velocity (3.4.04)
u_o	Carrier gas velocity at column outlet (3.6.05.2)
V	Volume in general
V_c	Column volume (3.2.05)
$V_{(DIE)}$	Volume of dry ion exchanger (5.6)
V_{ext}	Extra-column volume (6.1.03)
V_g	Specific retention volume at 0°C (3.7.11.2)
V_g^θ	Specific retention volume at column temperature (3.7.11.1)
V_i	Intraparticle volume of column in EC (6.1.02)
V_G	Interparticle volume of column in GC (3.2.11.2)
V_L	Liquid-phase volume (3.2.15)
V_M	Mobile-phase hold-up volume; except in EC (see 6.1.01) it is also equal to the retention volume of an unretained compound (3.7.03)
V_M^o	Corrected gas hold-up volume (3.7.04)
V_M	Volume of mobile phase in column (3.9.01)
V_N	Net retention volume (3.7.09)
V_o	Interparticle volume of column (3.2.11); in EC, it is also equal to the retention volume of an unretained compound (6.2.01)
V_R	Total retention volume (3.7.05; in EC: 6.2.02)
$\overline{V_R}$	Peak elution volume (3.7.06)
V'_R	Adjusted retention volume (3.7.07; in EC: 6.2.03)
V_R^o	Corrected retention volume (3.7.08)
V_S	Volume of stationary phase in column (3.2.15, 3.9.01)
$V_{(SIE)}$	Volume of swollen ion-exchanger (5.6)
$V_t^{(sol)}$	Total mobile-phase volume in the column (the mobile phase hold-up volume in EC) (6.1.03 and 6.2.04)
w_b	Peak width at base (3.3.07.1)
w_h	Peak with at half height (3.3.07.2)
w_i	Peak width at the inflection points (3.3.07.3)
W	Amount (mass) in general
W_i	Amount (mass) of a test substance present (4.2.01.2)
$W_{i(IE)}$	Amount of the component *i* in the ion exchanger (5.6)
$W_{i(M)}$	Amount of component *i* in the mobile phase (3.9)
$W_{i(S)}$	Amount of the component *i* in the stationary phase (3.9)
W_L	Amount (mass) of the liquid phase in the column (3.2.16)
W_S	Amount (mass) of the stationary phase in the column (3.2.16)
z	Number of carbon atoms of a *n*-alkane eluted before the peak of interest (3.7.15)
$(z+1)$	Number of carbon atoms of a *n*-alkane eluted after the peak of interest (3.7.15)

Greek symbols

α	Separation factor (relative retardation) (3.7.4.2)
α	Separation factor in IEC (5.5.04)
α_G	Unadjusted separation factor (relative retention) (3.7.14.3)
β	Phase ratio (3.2.17)
ε	Interparticle porosity (3.2.12)
η	Mobile phase viscosity (3.6.01)
θ	superscript in V_g^θ (3.7.11.1)
κ	log *k* (3.7.12.1)
υ	Reduced mobile phase velocity (3.6.05.3)
ρ	Bed density in IEC (5.6.03)
σ	Standard deviation of a Gaussian peak (3.3.06.5)
σ^2	Variance of a Gaussian peak (3.3.06.5)
Φ	Flow resistance parameter (3.2.19)

Subscripts

The generally used subscripts are listed. There are a few specific subscripts not listed here.

a	Ambient
c	Column
eff	Effective
f	Film of liquid phase
i	Compound of interest
o	Outlet of column
p	Particle
st	Standard

Table 2 *Continued*

Subscripts	
G	Gas phase
L	Liquid stationary phase
M	Mobile phase; also external solution in IEC
N	Net (as in net retention time or volume; correction for both the holdup time (volume) and gas compressibility)
R	Retention (as in retention time or volume)
S	Stationary phase; in IEC: ion exchanger
1,2	Two adjacent ($t_{R2} > t_{R1}$ except in EC where $M_2 > M_1$ and thus $t_{R1} > t_{R2}$
Superscripts	
T	Indication that value was obtained in programmed-temperature analysis
′	Adjusted (as in adjusted retention time or volume)
o	Corrected (as in corrected retention time or volume)

Table 3 List of acronyms used in chromatography

EC	Exclusion chromatography
GC	Gas chromatography
GLC	Gas-liquid chromatography
GLPC	Gas-liquid partition chromatography
GPC	Gel-permeation chromatography
GSC	Gas-solid chromatography
HETP	Height equivalent to one theoretical plate
HPLC	High-performance liquid chromatography
IC	Ion chromatography
IEC	Ion-exchange chromatography
LC	Liquid chromatography
LLC	Liquid-liquid chromatography
LSC	Liquid-solid chromatography
PC	Paper chromatography or Planar chromatography
PLOT	Porous-layer open-tubular (column)
PTV	Programmed-temperature vaporizer
RRT	Relative retention time
SCOT	Support-coated open-tubular (column)
SFC	Supercritical-fluid chromatography
TLC	Thin-layer chromatography
WCOT	Wall-coated open-tubular (column)

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5. Special Terminology Used in Ion-exchange Chromatography

The general terms and definitions discussed in the previous chapters are also valid in ion-exchange chromatography. In addition, the following terms and definitions refer specifically to this variant of the technique.

5.1. Basic Definitions

5.1.01. Ion exchange The process of exchanging ions between a solution and an ion exchanger.

5.1.02. Counter-ions In an ion exchanger, the mobile exchangeable ions.

5.1.03. Fixed ions In an ion exchanger, the non-exchangeable ions which have a charge opposite to that of the counter-ions.

5.1.04. Ion-exchange isotherm The concentration of a counter-ion in the ion exchanger expressed as a function of its concentration in the external solution under specified conditions and at constant temperature.

5.1.05. Sorption Uptake of electrolytes or non-electrolytes by the ion exchanger through mechanisms other than pure ion exchange.

5.1.06. Sorption isotherm The concentration of a sorbed species in the ion exchanger, expressed as a function of its concentration in the external solution under specified conditions and at constant temperature.

5.1.07. Ionogenic groups The fixed groupings in an ion exchanger which are either ionized or capable of dissociation into fixed ions and mobile counter-ions

5.1.08. Co-ions The mobile ionic species in an ion exchanger with a charge of the same sign as the fixed ions.

5.1.09. Cation exchange The process of exchanging cations between a solution and a cation exchanger.

5.1.10. Anion exchange The process of exchanging anions between a solution and an anion exchanger.

5.2. The Mobile Phase

5.2.01. Solvent The term used in classical ion exchange to express the mobile phase.

5.2.02. External solution The solution in contact with the ion exchanger which contains the ionized species before and after exchange with the ion exchanger.

5.3. The Chromatographic Medium

5.3.01. Ion exchangers A solid or liquid, inorganic or organic substance containing ions exchangeable with others of the same charge, present in a solution in which the ion exchanger is considered to be insoluble.

Note: It is recognized that there are cases where liquid exchangers are employed and where it may be difficult to distinguish between the separation process as belonging to ion exchange or liquid-liquid distribution, but the broad definition given here is regarded as that which is most appropriate.

5.3.01.1. Resin matrix The molecular network of an ion exchanger which carries the ionogenic groups.

5.3.01.2. Monofunctional ion exchanger An ion exchanger containing only one type of ionogenic group.

5.3.01.3. Bifunctional ion exchanger An ion exchanger containing two types of ionogenic groups.

5.3.01.4. Polyfunctional ion exchanger An ion exchanger containing more than one type of ionogenic groups.

5.3.01.5. Macroporous ion exchanger An ion exchanger with pores that are large compared to atomic dimensions.

5.3.01.6. Salt form of an ion exchanger The ionic form of an ion exchanger in which the counter-ions are neither hydrogen nor hydroxide ions. When only one valence is possible for the counter-ion, or its exact form or charge is not known, the symbol or the name of the counter-ion without charge is used, e.g., sodium-form or Na-form, tetramethylammonium-form, orthophosphate-form. When one of two or more possible forms is exclusively present, the oxidation state may be indicated by a Roman numeral, e.g. Fe^{II}-form, Fe^{III}-form.

5.3.01.7. Redox polymers Polymers containing functional groups which can be reversibly reduced or oxidized. *Electron Exchanger* may be used as a synonym.

5.3.01.8. Redox ion exchangers Conventional ion exchangers in which reversible redox couples have been introduced as counter-ions either by sorption or complex formation. They closely resemble redox polymers in their behavior.

5.3.02. Cation exchanger Ion exchanger with cations as counter-ions. The term *Cation-Exchange Resin* may be used in the case of solid organic polymers.

5.3.02.1. Acid form of a cation exchanger The ionic form of a cation exchanger in which the counter-ions are hydrogen ions (H-form) or the ionogenic groups have added a proton forming an undissociated acid.

5.3.03. Anion exchanger Ion exchanger with anions as counter-ions. The term *Anion-Exchange Resin* may be used in the case of solid organic polymers.

5.3.03.1. Base form of an anion exchanger The ionic form of an anion exchanger in which the counter-ions are hydroxide groups (OH-form) or the ionogenic groups form an uncharged base, e.g. $-NH_2$.

5.3.04. Ion-exchange membrane A thin sheet or film of ion-exchange material which may be used to separate ions by allowing the preferential transport of either cations (in the case of a *Cation-Exchange Membrane*) or anions (in the case of an *Anion-Exchange Membrane*). If the membrane material is made from only ion-exchanging material, it is called a *Homogeneous Ion-Exchange Membrane*. If the ion-exchange material is embedded in an inert binder, it is called a *Heterogeneous Ion-Exchange Membrane*.

5.3.04.1. Perm-selectivity A term used to define the preferential permeation of certain ionic species through ion-exchange membranes.

5.3.05. Weight-swelling ratio in solvent Mass of solvent taken up by unit mass of the dry ion exchanger. The solvent must always be specified.

5.3.06. Volume-swelling ratio Ratio of the dry swollen volume to the true dry volume of the ion exchanger.

5.4. Capacity Values

5.4.01. Theoretical specific capacity Amount (mmol) of ionogenic group per mass (g) of dry ion exchanger. If not otherwise stated, the capacity should be reported per mass (g) of the H-form of a cation exchanger and of the Cl-form of an anion exchanger.

5.4.02. Volume capacity (Q_v) Amount (mmol) or ionogenic group per volume (cm^3) of swollen ion exchanger. The ionic form of the ion exchanger and the medium should be stated.

5.4.03. Bed volume capacity Amount (mmol) of ionogenic group per bed volume (cm^3) (see 3.2.06) determined under specified conditions. The conditions should always be specified.

5.4.04. Practical specific capacity (Q_A) Total amount of ions (mmole) taken up per mass (g) of dry ion exchanger under specified conditions. The conditions should always be specified.

5.4.05. Break-through capacity of ion-exchange bed (Q_B) The practical capacity of an ion exchanger bed, obtained experimentally by passing a solution containing a particular ionic or molecular species through a column containing the ion exchanger. This is under specified conditions and is determined by measuring the amount of species which has been taken up when the species is first detected in the effluent or when the concentration in the effluent reaches some arbitrarily defined value. The break-through capacity may be expressed in millimoles or milligrams taken up per gram of dry ion exchanger of per cm^3 of bed volume.

5.5. Diffusion, Selectivity and Separation

5.5.01. Diffusion coefficient in the ion exchanger (D_{ex}) The meaning of this term is the same as the specified in 3.4.01-3.4.02.

5.5.02. Selectivity coefficient ($k_{A/B}$) The equilibrium coefficient obtained by application of the law of mass action to ion exchange and characterizing quantitatively the ability of an ion exchanger to select one of two ions present in the same solution. The ions involved in the exchange should be specified as subscripts. Examples:

Exchange: $Mg^{2+} - Ca^{2+}$

$$k_{Mg/Ca} = \frac{[Mg]_S/[Ca]_S}{[Mg]_S/[Ca]_S}$$

Exchange: $SO^{2+} - Cl^{-}$

$$k_{SO_4/Cl} = \frac{[SO_4]_S/[Cl]_S^2}{[SO_4]_M/[Cl]_M^2}$$

In the above equations subscript S refers to the ion exchanger ('stationary phase') and M to the external solution ('mobile phase'). For exchanges involving counter-ions differing in their charges, the numerical value of $k_{A/B}$ depends on the choice of the concentration scales in the ion exchanger and the external solution (molal scale, molar scale, mole fraction scale, etc.). Concentration units must be clearly stated for an exchange of ions of differing charges.

5.5.03. Corrected selectivity coefficient ($k^a_{A/B}$) This is calculated in a way identical to the selectivity coefficient except that the concentrations in the external solutions are replaced by activities.

5.5.04. Separation factor ($\alpha_{A/B}$) The definition of this term is identical to the definition given in 3.7.14.2. In an exchange of counter-ions of equal charge the separation factor is equal to the selectivity coefficient (see 5.5.01), provided that only one type of ion represents the analytical concentration (e.g. in exchanges of K^+ and Na^+) but not in systems where several individual species are included in the analytical concentrations.

5.6. Distribution constants

A *Distribution Constant* is the concentration of a component in the ion exchanger (the stationary phase) divided by its concentration in the external solution (the mobile phase). The concentration in the external solution is always calculated per unit volume. Depending on the way the concentration in the ion exchanger is expressed three forms of the distribution constant may exist.

In 5.6.01-5.6.03, $W_{i(IE)}$ and $W_{i(sol)}$ are the amounts of the component i in the ion exchanger and in the external solution; V_{SIE} and $_{DIE}$ are the volumes of the swollen and dry ion exchanger, respectively; $V_{(sol)}$ is the volume of the external solution.

5.6.01. Distribution constant (K_c) In this case, the concentration in the ion exchanger is calculated as mass (weight)/volume and it refers to the swollen ion exchanger:

$$K_c = \frac{W_{i(IE)}/V_{(SIE)}}{W_{i(sol)}/V_{(sol)}}$$

5.6.02. Distribution constant (K_g) In this case, the concentration in the ion exchanger is calculated as mass/mass (weight/weight) and it refers to dry ion exchanger:

$$K_g = \frac{W_{i(IE)}/V_{(DIE)}}{W_{i(sol)}/V_{(sol)}}$$

5.6.03. Distribution constant (K_v) In this case, the concentration in the ion exchanger is calculated as volume/volume and it refers to the dry ion exchanger:

$$K_v = \frac{V_{i(IE)}/V_{(DIE)}}{W_{i(sol)}/V_{(sol)}}$$

If the *Bed Density* is ρ, expressed in grams of dry resin per cm^3 of bed, then

$$K_v = K_g \rho$$

6. Special Terminology Used in Exclusion Chromatography

Besides the terms and definitions used in general in chromatography, a number of special terms exist in exclusion chromatography. In addition, due to the different nature of the chromatographic separation, some

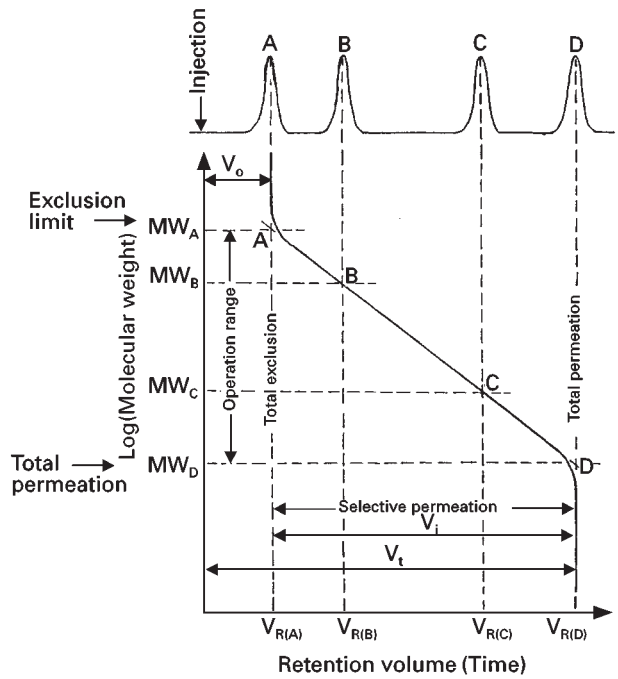

Figure 7 Retention characteristics in exclusion chromatography. A standard sample in analysed (top); subsequently, the retention volumes (times) are plotted against the logarithms of the corresponding molecular weights. Peak A corresponds to a non-retained sample component the molecules of which are larger than the largest pores in the gel particles (total exclusion); peak D corresponds to a sample component the molecules of which are smaller than the smallest pores in the gel particles (total penetration).

of the general chromatographic terms have a different meaning here. For further explanation of some of the terms, *see* **Figure 7**.

Below, only the *chromatography* terms are listed. For a discussion of the molecular weight terms calculated from the chromatographic data see the specialized nomenclatures (e.g. refs. 15–18).

6.1. The Column

6.1.01. Interparticle volume of the column (V_o) The volume of the mobile phase in the interstices between the gel particles. It is also called the *Interstitial Volume* of the column.

In exclusion chromatography, the interparticle volume of the column is equal to the retention volume of an unretained compound; however, it is *not* equal to the mobile phase hold-up volume (V_t). The reason for this is that is practice the mobile phase molecules are always smaller than the smallest pores of the column packing. Thus, they will enter all the pores available in the packing and therefore, will be eluted last. As a contrast, in general liquid chromatography, the mobile-phase hold-up volume (see 3.7.03) and the retention volume of a non-retained compound are practically equal.

6.1.02. Intraparticle volume of the column (V_i) The volume of the mobile phase within the pores of the gel particles. It is also called the *Intrastitial Volume* of the column or the *Stationary Mobile-Phase Volume*.

The retention time equivalent to V_i is t_i:

$$t_i = V_i/F_c$$

6.1.03. Total mobile-phase volume in column (V_t) The sum of the interparticle and intraparticle volumes:

$$V_t = V_o + V_i$$

In the definition of V_t the extra-column volume of the system (V_{ext}; see 3.2.13) is neglected. If it is not negligible, it must also be added:

$$V_t = V_o + V_i + V_{ext}$$

6.2. Retention Parameters

6.2.01. Retention volume (time) of an unretained compound (V_o, t_o) The retention volume of a sample component the molecules of which are larger than the largest pores of the gel particles. These will be eluted first from the column. The corresponding retention time is t_o:

$$t_o = V_o/F_c$$

Ignoring any extra-column volume, V_o is equal to the interparticle volume of the column (see 6.1.01).

6.2.02. Retention volume (time) (V_R, t_R) The retention volume (time) of a sample component the molecules of which are smaller than the largest pores of the gel particles but larger than the smallest pores. The corresponding retention time is t_R:

$$t_R = V_R/F_c$$

6.2.03. Adjusted retention volume (time) (V'_R, t'_R) The total retention volume less the retention volume of an unretained compound:

$$V'_R = V_R - V_o$$

The corresponding retention time is t'_R:

$$t'_R = t_R - t_o = V'_R/F_c = (V_R - V_o)/F_c$$

6.2.04. Total mobile phase volume (time) (V_t, t_t) The retention volume (time) of a sample component the molecules of which are smaller than the smallest pores of the gel particles. The corresponding retention times is t_t:

$$t_t = V_t/F_c$$

6.2.05. Retention factor (k_e) The ratio of the adjusted retention volume (time) and the retention volume (time) of an unretained compound:

$$k_e = \frac{V_R - V_o}{V_o} = \frac{t_R - t_o}{t_o}$$

It may also be called the *Capacity Factor*. However, the suggested expression better defines its real meaning (see also 3.7.12).

6.2.06. Distribution constant in exclusion chromatography (k_o) The fraction of the intraparticle volume (the volume of the pores) available to the molecules of a particular sample component for diffusion:

$$K_o = \frac{V_R - V_o}{V_i}$$

For an unretained compound, $V_R = V_o$ and thus, $K_o = 0$. On the other hand, for a compound the molecules of which are smaller than the smallest pores, $V_R = V_t$ and thus, $K_o = 1$. In other words, the value of K_o varies between zero and unity.

In exclusion chromatography, K_o is related to the retention volume of a sample component and the inter- and intraparticle volumes of the column (V_o and V_i, respectively) in a manner analogous to the relationship in general liquid chromatography:

$$V_R = V_o + K_o V_i$$

6.3. Efficiency Terms

6.3.01. Peak resolution ($R_{1/2}$) The definition of this term is identical to that given in 3.10.01.

$$R_{1/2} = \frac{V_{R1} - V_{R2}}{(w_{b1} + w_{b2})/2}$$

Here V_{R1} and V_{R2} represent the peaks corresponding to compounds with molecular masses M_1 and M_2 respectively: by definition $M_2 > M_1$. In exclusion chromatography, larger molecules are eluted first, therefore, $V_{R1} > V_{R2}$.

Because of the addition of a new term, the specific resolution (see 6.3.02), the symbol $R_{1/2}$ is suggested for peak resolution in exclusion chromatography.

6.3.02. Specific resolution (R_{sp}) Peak resolution also considering the molecular masses of the two test compounds:

$$R_{sp} = \frac{V_{R1} - V_{R2}}{(w_{b1} + w_{b2})/2} \frac{1}{\log(M_2/M_1)}$$

The test compounds used for the determination of the specific resolution should have a narrow molecular-mass distribution (the ratio of the mass-average and number-average molecular masses should be equal to or less than about 1.1) and differ by a factor of about 10 in their molecular masses.

Note: In some nomenclatures, the symbol R_s is used for the specific resolution. Due to the possibility of confusing it with the general resolution term (see 3.10.01), the symbol R_{sp} is suggested here.

6.3.03. Plate number and plate height (N, H) The definitions of these terms are identical to those given in 3.10.03 and 3.10.05.

6.3.04. Effective plate number and effective plate height (N_{eff}, H_{eff}) The definitions of these terms are identical to those given in 3.10.04 and 3.10.06, except that the retention volume of a non-retained compound (V_o; see 6.2.01) is used in the calculations:

$$N_{eff} = 16\left[\frac{V_R - V_o}{w_b}\right]^2 = 5.545\left[\frac{(V_R - V_o)}{w_h}\right]^2$$

$$N_{eff} = 16\left[\frac{t_R - t_o}{w_b}\right]^2 = 5.545\left[\frac{(t_R - t_o)}{w_h}\right]^2$$

$$H_{eff} = L/N_{eff}$$

6.3.05. Reduced plate height (h) The definition of this term is identical to that given in 3.10.07.

12B. Liquid-Liquid Distribution (Solvent Extraction) (IUPAC Recommendations 1993)

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