IUPAC Recommendations

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Terminology of separation methods (IUPAC Recommendations 2017)

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Abstract: Recommendations are given concerning the terminology of methods of separation in analytical chemistry, including chromatography, electromigration techniques, and field-flow fractionation and related techniques.

Keywords: chromatography; electromigration; field-flow fractionation; separation science.

CONTENTS

1 Introduction

This chapter contains terms and definitions of concepts relating to analytical aspects of separation. Most of the terms have been drawn from papers published in Pure and Applied Chemistry. A number of new sections and terms have been included, using terms proposed as definitions in the literature. To complete these areas, a number of new terms have been proposed for acceptance.

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The capitalization of previously accepted terms has been corrected to bring them up to date with current practice and terms have also been amended, in certain cases, to link the definitions specifically to chromatography "(in chromatography)". In a few cases, minor changes have been made to include both LC and GC in a term, or to reflect significant changes in practice, such as the universal change from chart recorders to electronic integration. Terms included without change from previous PAC Recommendations are cited with the original number, *e.g.* Source: [1] 1.1.01. Those that have minor changes are noted, *e.g.* Source: [1] 1.1.01 (with minor change), as are those with changes based on any Source, *e.g.* Source: Adapted from [1].

Terms in italics refer to terms defined within this paper and appear in italics on first use in a subsection or definition.

This Recommendation is part of the update of the Orange Book [2] and will be the basis for a chapter in the forthcoming fourth edition.

2 Chromatography

2.1 Basic terms and definitions

2.1.1 bonded stationary phase (material) (in chromatography)

bonded stationary phase
bonded phase (material)

Stationary phase which is covalently bonded to solid support particles or to the inside wall of the *column*.

Note 1: The *bonded stationary phase* (material) may be monomeric, polymeric or polymer-grafted phase (material) and the stationary phase (material) can also receive additional treatment to give a capped (end-capped) stationary phase (material).

Source: [3] 3.1.2

2.1.2 chromatogram

Graphical or other presentation of *detector* response against *effluent* volume or time in *chromatography*.

Note 1: In *planar chromatography* "chromatogram" may refer to the paper or layer with zones containing separated components of the sample.

Source: Adapted from [1] 1.1.02

2.1.3 chromatograph (noun)

Instrument for carrying out *chromatography*.

Source: [1] 1.1.04

2.1.4 chromatograph (verb)

To separate by *chromatography*.

Source: [1] 1.1.03

2.1.5 chromatographic bed

The form in which the *stationary phase* is used.

2.1.6 chromatography

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 (*mobile phase*) moves in a definite direction.

Source: [1] 1.0.01

2.1.7 effluent (in chromatography)

Mobile phase leaving the *column*.

Source: [1] 1.1.08

2.1.8 immobilized stationary phase (material) (in chromatography)
immobilized stationary phase

Stationary phase which has been immobilized on the support particles or on the inner wall of the *column*, *e.g.* by a physical attraction (coated stationary phase), by chemical bonding (*bonded stationary phase*), or by *in situ* polymerisation (cross-linked stationary phase) after coating.

Source: [3] 3.1

2.1.9 mobile phase (in chromatography)

Fluid which percolates through or along the *stationary phase*, in a definite direction.

Note 1: The mobile phase may be a liquid (*liquid chromatography*), a gas (*gas chromatography*), or a supercritical fluid (*supercritical fluid chromatography*). In gas chromatography, the term "carrier gas" may be used for the mobile phase. In *elution chromatography,* the term "eluent" is also used for the mobile phase.

Source: [1] 1.1.06 (with minor change)

2.1.10 stationary phase (in chromatography)

Part of a chromatographic system responsible for the retention of the analytes, which are being carried through the system by the *mobile phase*.

Note 1: The stationary phase may be a solid, a gel, or a liquid. If a liquid, it may be distributed on a solid support. This solid support 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).

Note 2: The term *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 3: 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' has 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 stationary phase* or *immobilized stationary phase*, are recommended.

Source: [3] 2.1

2.1.11 zone (in chromatography)
band

Region in the *chromatographic bed* where one or more components of the analytical sample are located.

Source: [1] 1.1.13

2.2 Principal methods

2.2.1 displacement chromatography

Chromatography in which the *mobile phase* contains a compound (the displacer) more strongly retained than the components of the sample under examination.

Note 1: The sample is fed into the system as a finite slug.
Source: [1] 1.2.02 (with minor change)

2.2.2 elution chromatography

Chromatography in which the *mobile phase* is passed through the *chromatographic bed* after the introduction of the sample.

Source: [1] 1.2.03 (with minor change)

2.2.3 frontal chromatography

Chromatography in which the analytical sample (liquid or gas) is fed continuously into the *chromatographic bed*.

Note 1: In frontal chromatography no additional *mobile phase* is used.
Source: [1] 1.2.01 (with minor change)

2.3 Classification according to the shape of the *chromatographic bed*

2.3.1 column chromatography

Chromatography in which the *chromatographic bed* is within a tube.

Note 1: The particles of the solid stationary phase or 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*).
Source: [1] 1.3.01 (with minor change)

2.3.2 planar chromatography

Chromatography in which the *stationary phase* is present as, or on, a plane.

Note 1: The plane can be a paper, which may serve as, or be impregnated by a substrate serving as, the stationary bed (*paper chromatography*), or it can be a layer of solid particles spread on a support, *e.g.* a glass or metal plate (*thin-layer chromatography*, TLC).
Source: [1] 1.3.02 (with minor change)

2.4 Classification according to the physical state of the mobile phase

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)
- *supercritical fluid chromatography* (SFC)

The term Gas-Liquid Partition Chromatography (GLPC) can also be found in the literature. However, distinguishing between these modes is often difficult. For example, in GC, a liquid may be used to modify an adsorbent-type solid stationary phase.

2.4.1 gas chromatography (GC)

Column chromatography in which the *mobile phase* is a gas.

Note 1: Gas chromatography is always carried out in a *column*.
Source: [1] 1.4.02 (with minor change)

2.4.2 liquid chromatography (LC)

Chromatography in which the *mobile phase* is a liquid.

Note 1: Liquid chromatography can be carried out either in a *column* or on a plane.
Note 2: Liquid column chromatography utilizing small particles (2–20 μm) and a relatively high inlet pressure (up to 50 MPa (500 bar)) is often characterized by the term high-performance liquid chromatography, and the acronym HPLC.
Note 3: If smaller particles (less than 2 μm) and higher inlet pressures (up to 150 MPa (1500 bar)) are employed, then the method is characterized by the term ultra-high-performance liquid chromatography UHPLC) or ultra-performance liquid chromatography (UPLC).
Source: [1] 1.4.03 (with minor change)

2.4.3 supercritical fluid chromatography (SFC)

Chromatography in which the *mobile phase* is a fluid above and relatively close to its critical temperature and pressure.

Note 1: In general, the terms and definitions used in *gas chromatography* or *liquid chromatography* are equally applicable to supercritical fluid chromatography.
Source: [1] 1.4.04 (with minor change)

2.5 Classification according to the mechanism of separation

2.5.1 adsorption chromatography

Chromatography in which separation is based mainly on differences between the adsorption affinities of the sample components for the surface of an active solid as the *stationary phase*.

Source: [1] 1.5.01

2.5.2 affinity chromatography

Chromatography in which the separation is based on the unique biological specificity of the interaction between analyte and stationary ligand.

Source: Adapted from [1] 1.5.05

2.5.3 counter-current chromatography (CCC)

Partition chromatography which uses two immiscible liquid phases and no solid support. One liquid acts as the *stationary phase* and the other as the *mobile phase*.

Note 1: The method employs a variety of systems using either gravity or centrifugal force to retain the stationary phase in the *column*.
Note 2: The method allows continuous elution of the mobile phase and on-line monitoring of the *effluent*.

Source: [4]

2.5.4 enantioselective chromatography (electrophoresis)
chiral chromatography (electrophoresis)

Separation of enantiomeric species due to the enantioselectivity of their interaction with the chiral selector(s) of a chromatographic (electrophoretic) system.

Source:[5]

2.5.5 exclusion chromatography

Chromatography in which separation is based mainly on exclusion effects, such as differences in molecular size and/or shape or charge.

Note 1: The terms gel filtration and gel-permeation chromatography (GPC) were used earlier to describe this process when the stationary phase was a swollen gel.
Source: [1] 1.5.04 (with minor changes)

2.5.6 hydrodynamic chromatography

Liquid chromatography for the separation of macromolecules and particles based on the difference in the speed of the *mobile phase* flow between the *column* axis and column wall and the specific size-dependent distribution of particles across the column cross-section.

2.5.7 hydrophilic-interaction liquid chromatography (HILIC)

Liquid chromatography in which the analytes are partitioned between a water-enriched layer, which is immobilised on a hydrophilic (polar) *stationary phase*, and a less-polar *mobile phase*, containing water and organic solvent.

Source: [6]

2.5.8 hydrophobic-interaction chromatography (HIC)

Chromatography in which molecules are separated by interactions between their hydrophobic moieties and hydrophobic sites on the surface of the *stationary phase*.

Note 1: High salt concentrations are used in the *mobile phase* and separations are effected by changing the salt concentration. Gradients are run by decreasing the salt concentration over time.

Source: Adapted from [7]

2.5.9 ion-exchange chromatography
ion chromatography (IC)

Chromatography in which separation is based mainly on differences in the ion-exchange affinities of the sample components.

Note 1: IC utilizes small-particle high-efficiency *column*s and usually conductometric or spectroscopic detectors.

Source: [1] 1.5.03 (with minor changes)

2.5.10 ion-exclusion chromatography

Exclusion chromatography in which ionized samples are excluded from the pores of the support and elute first, while weakly ionized and non-ionic compounds elute later.

2.5.11 ion-pair chromatography

Liquid chromatography in which the *mobile liquid* phase contains an ionized additive (an ion-pair reagent) that can form an *ion pair* with an analyte molecule.

Note 1: Separations occur due to the difference in the ability to form ion pairs and/or in the distribution coefficients of the ion pairs between the mobile and stationary phases.

2.5.12 ligand exchange chromatography

Chromatography based on the different ability of substances to form complexes with metal ions.

2.5.13 micellar chromatography

Reversed-phase liquid chromatography which uses a *mobile phase* containing a surfactant above its critical micellar concentration (cmc).

2.5.14 normal-phase chromatography

Liquid chromatography in which the *stationary phase* is more polar than the *mobile phase*.

Note 1: This term is used in liquid chromatography to emphasize the contrast to *reversed-phase chromatography*.
Example: Mobile phase is chloroform/ethyl acetate and the stationary phase is silica.
Source: [1] 1.6.02 (with minor changes)

2.5.15 partition chromatography

Chromatography in which separation is based mainly on differences between the solubility of the sample components in the *stationary phase* (*gas chromatography*), or on differences between the solubilities of the components in the *mobile phase* and stationary phase (*liquid chromatography*).

Source: [1] 1.5.02

2.5.16 pyrolysis-gas chromatography

Chromatography in which an analytical sample is thermally decomposed to smaller fragments before entering the *column*.

Source: [1] 1.6.11.1 (with minor change)

2.5.17 reversed-phase chromatography
deprecated: reverse-phase chromatography

Liquid chromatography in which the *mobile phase* is significantly more polar then the *stationary phase*.

Example: Mobile phase is water/methanol, and stationary phase is a silica-based material with chemically bonded alkyl chains.
Source: [1] 1.6.01

2.5.18 size-exclusion chromatography

Exclusion chromatography in which separation is based mainly on differences in molecular size.

2.6 Classification according to the mode of elution

2.6.1 gradient elution (in chromatography)

Elution in which the composition of the *mobile phase* is changed continuously or stepwise.

Source: [1] 1.6.04

2.6.2 isocratic elution (in chromatography)
isocratic analysis

Chromatography in which the composition of the *mobile phase* remains constant during *elution*.

Source: [1] 1.6.03

2.6.3 isothermal chromatography

Chromatography in which the temperature of the *column* is kept constant during the separation.

Source: [1] 1.6.07

2.6.4 post-column derivatization (in chromatography)

Technique in *chromatography* in which the separated components eluting from the *column* are chemically derivatized before entering the *detector*.

Note 1: Derivatization is generally carried out "on-the-fly", *i.e.* during transfer of the sample components from the column to the detector.

Note 2: Post-column derivatization provides molecules with properties that improve detection. For example, trialkylamines react with tris-(2,2′-bipyridyl)ruthenium (III) to produce short-lived chemiluminescence.

See also: *pre-column derivatization*

Source: [1] 1.6.11.2 (with minor changes)

2.6.5 pre-column derivatization (in chromatography)

Technique in *chromatography* in which the sample is chemically derivatized before entering the *column*.

See also: *post-column derivatization*

Source: Adapted from [1] 1.6.11.2

2.6.6 programmed-flow chromatography
flow programming

Chromatography in which the *flow rate* of the *mobile phase* is changed systematically during a part of or the whole of the separation.

Source: [1] 1.6.09

2.6.7 programmed-pressure chromatography
pressure programming

Chromatography in which the inlet pressure of the *mobile phase* is changed systematically during a part of or the whole of the separation.

Source: [1] 1.6.10

2.6.8 programmed-temperature chromatography
temperature programming

Chromatography in which the temperature of the *column* is changed systematically during a part of or the whole of the separation.

Example: In the analysis of polymers, temperature rising elution fractionation (TREF) is used to characterize short-chain copolymers [8].

Source: [1] 1.6.08

2.6.9 stepwise elution (in chromatography)

Gradient elution in which the composition of the mobile phase is changed in steps during a single chromatographic run.
Source: [1] 1.6.05

2.7 Terms related to the chromatographic process and the theory of chromatography

The Column

2.7.1 capillary column (in chromatography)

General term for *columns* having a small diameter.

Note 1: A capillary column may contain a packing or have the stationary phase supported on its inside wall. The former case corresponds to a *packed column*, while the latter case corresponds to an *open-tubular column*.
Note 2: Due to the ambiguity of this term its use without an adjective is discouraged.
Source: [1] 3.2.04

2.7.2 column diameter, d_c

The inner diameter of the *column* tubing

Note 1: The column radius $r_c = d_c/2$.
Source: [1] 3.2.08

2.7.3 column (in chromatography)

Tube and *stationary phase* contained within, through which the *mobile phase* passes.

Source: [1] 3.2.01

2.7.4 column length, L

The length of that part of the *column* tube that contains the *stationary phase*.

Source: [1] 3.2.09

2.7.5 column volume, V_c

Volume of the part of the *column* tube that contains the packing, calculated as the product of the *internal cross-sectional area of the column* A_c and the length of the packed part of the column L. $V_c = A_c \times L$.

Note 1: 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.
Source: [1] 3.2.05

2.7.6 cross-sectional area of the column, A_c

The cross-sectional area of the empty tube: $A_c = \pi r_c^2 = \pi(d_c/2)^2$, where d_c is the *column diameter*.
Source: [1] 3.2.10

2.7.7 extra-column volume (time) (in chromatography), V_{ext}, t_{ext}

deprecated term: dead-volume

Volume (time taken by a component) between the effective injection point and the effective detection point, excluding the part of the column containing the *stationary phase*.

Note 1: V_{ext} is composed of the volumes of the injector, connecting lines, and detector.
Note 2: 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.
Source: [1] 3.2.13

2.7.8 interparticle porosity (in chromatography), ε

interstitial fraction

Interparticle volume (V_0) of a *packed column* divided by *column volume* (V_c):

$$\varepsilon = V_0/V_c$$

Source: [1] 3.2.12

2.7.9 interparticle volume of the column (in chromatography), V_0

interstitial volume
void volume

Volume occupied by the *mobile phase* between particles in the packed section of a *column*.

Note 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.
Note 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^0): $V_G = V_M^0$

Source: [1] 3.2.11

2.7.10 liquid-phase film thickness, d_f

Average thickness of the liquid *stationary phase* film coated on the inside wall of the *column* tubing of an *open-tubular column*.

Source: [1] 3.2.14

2.7.11 mass of the stationary phase (in chromatography), m_S

The mass of the liquid *stationary phase* or the active solid in the *column*.

Note 1: The mass of any solid support is not included.
Note 2: In *partition chromatography* with a liquid stationary phase, m_S is identical to the liquid phase mass (m_L).
Source: [1] 3.2.16 (with minor change)

2.7.12 monolithic column

Column packed with a single piece of solid material (monolith) composed of interconnected skeletons (forming macropores) and interconnected flow paths (mesopores) as a *stationary phase* material.

2.7.13 open-tubular column

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.

Note 1: If the column is a *capillary column,* it is termed an open-tubular capillary column.
Source: Adapted from [1] 3.2.03

2.7.14 packed column

Column containing solid packing.

Note 1: If the column is a *capillary column,* it is termed a packed capillary column.
Source: [1] 3.2.02 (with minor changes)

2.7.15 phase ratio (in chromatography), β

Ratio of the volume of the *mobile phase* (V_0) to that of the the *stationary phase* (V_S) in a *column*: $\beta = V_0/V_S$

Note 1: In the case of *open-tubular columns* V_0 is substituted by the geometric internal volume of the tube (V_c).
Source: [1] 3.2.17

2.7.16 porous-layer open-tubular (PLOT) column

Open-tubular column with a porous layer on the inner wall.

Note 1: Porosity can be achieved by either chemical means (*e.g.* etching) or by the deposition of porous particles on the wall from a suspension.
Note 2: The porous layer may serve as a support for a liquid stationary phase or as the stationary phase itself.
Source: [1] 3.2.03.2 (with minor changes)

2.7.17 stationary-phase volume, V_S

Volume of the liquid *stationary phase* or the active solid in the *column*.

Note 1: The volume of any solid support is not included.
Note 2: In *partition chromatography* with a liquid stationary phase, V_S is identical to the liquid-phase volume (V_L).
Source: [1] 3.2.15 (with minor change)

2.7.18 support-coated open-tubular (SCOT) column

Type of *porous-layer open-tubular column* in which the porous layer consists of support particles deposited from a suspension.

Source: Adapted from [1] 3.2.03.3

2.7.19 wall-coated open-tubular (WCOT) column

Open-tubular column in which the liquid *stationary phase* is coated on the essentially unmodified smooth inner wall of the tube.
Source: [1] 3.2.03.1

The Chromatogram

2.7.20 asymmetry factor (in chromatography), A_s
asymmetry

Width of a *peak* ($f+b$) divided by twice the width of the front of the peak (f), measured at 10 % of the *peak height* (Fig. 1)

$$A_s = \frac{(f+b)}{2f}$$

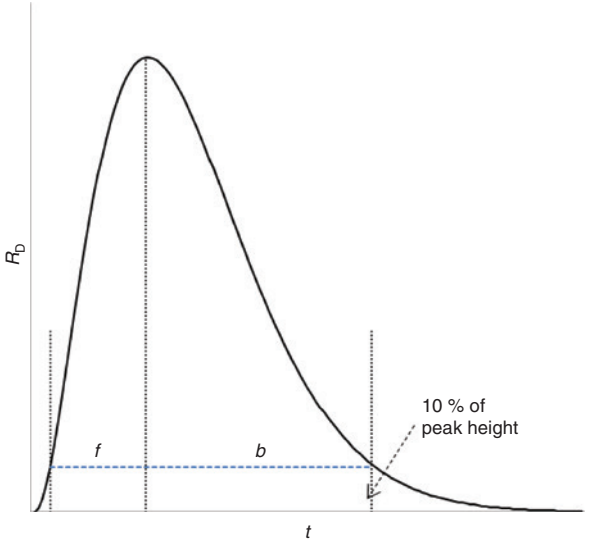

Fig. 1: Peak asymmetry (schematic). R_D is the response of the *detector*.

Note 1: For a symmetrical peak $A_s = 1$.
Note 2: Some pharmacopoeia definitions of peak asymmetry measure the value at 5 % of the peak height.
See also *tailing factor*.
Source: [9]

2.7.21 asymmetry (in chromatography)

Deviation of the shape of a *peak* from an ideal Gaussian curve.

Note 1: Asymmetry is usually seen as tailing, when the width of the back of the peak is greater than the width of the front of the peak.

Note 2: Asymmetry is quantified as the *asymmetry factor* or *tailing factor*.

2.7.22 baseline (in chromatography)

Sections of a *chromatogram* recording the *detector* response when only *mobile phase* elutes from the *column*.

Note 1: The term baseline is also used for the constructed *peak base*.
Source: Adapted from [1] 3.3.05

2.7.23 peak (in chromatography)

Section of a *chromatogram* recording the *detector* response when one or more components elute from the *column*.

Note 1: The aim is to completely separate all components of interest, but if separation is incomplete, two or more components may be eluted as overlapping peaks or one unresolved peak.

Source: Adapted from [1] 3.3.06

2.7.24 peak base (in chromatography)

Line joining points on the *baseline* either side of a peak.

Note 1: The peak base may be calculated as part of a mathematical analysis of the peak.

2.7.25 peak height (in chromatography)

Distance between the *peak* maximum and *peak base*, measured in a direction parallel to the axis representing *detector* response.

Note 1: The peak height is shown in Fig. 2
Source: [1] 3.3.06.4

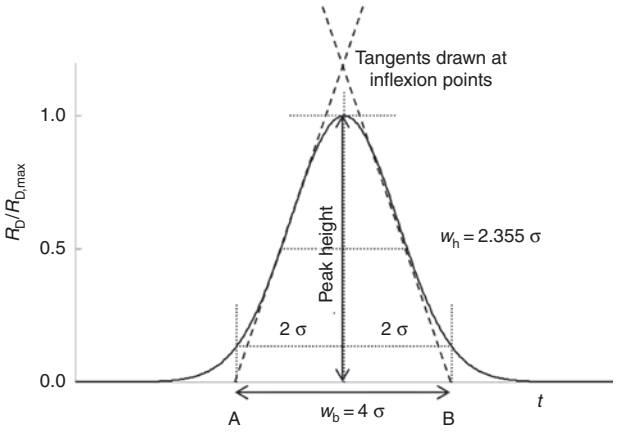

Fig. 2: Schematic of chromatographic peak showing the widths of a Gaussian peak at various peak heights as a function of the standard deviation of the modelled Gaussian. $R_D/R_{D,max}$ is the relative *detector* response.

2.7.26 peak width at base (in chromatography), w_b
base width

Peak width at *peak base* intercepted by tangents to the *chromatogram* drawn at the inflection points on either side of the peak. Represented by the line AB in Fig. 2

Source: Adapted from [1] 3.3.07.1, See Fig. 2

2.7.27 peak width at half height (in chromatography), w_h
deprecated term: half-width
deprecated symbol: $w_{1/2}$

Peak width at 50 % of the *peak height* that terminates at the intersections with the peak.

Note 1: The peak width at half height must never be called the "half width" because that has a completely different meaning.
Note 2: The peak width at half height of a Gaussian peak is about 2.355 times the standard deviation of the modelled Gaussian. See Fig. 2.
Source: Adapted from [1] 3.3.07.2

2.7.28 peak width (in chromatography)

Retention dimension (time or volume) parallel to the baseline of a *peak*.

Note 1: Peak widths are measured parallel to the axis representing time or volume, even if the baseline is not parallel to this axis.
Note 2: Two peak-width values are commonly used in chromatography, *peak width at base* and *peak width at half height*.
Source: Adapted from [1] 3.3.07

2.7.29 tailing factor (in chromatography)

Width of the back of a *peak* (*b*) divided by the width of the front (*f*) of the peak measured at 10 % of the *peak height*. (See Fig. 1).

Note 1: See Fig. 1, where tailing factor = b/f.
Source: [9]

The Mobile Phase

2.7.30 flow rate (in chromatography), F_c

The volume flow rate of the *mobile phase* passing through a *column*.

Source: Adapted from [1] 3.6.04

2.7.31 mobile-phase velocity (mobile-phase speed), *u* (*u*)

Linear velocity (speed) of the *mobile phase* across the average cross-section of the *column*.

Note: The velocity is defined perpendicular to the cross section of the tube in the direction of flow.
Source: [1] 3.6.05.1

Retention Parameters

2.7.32 adjusted retention volume (time), $V'_R(t'_R)$

Total retention volume (time) minus hold-up volume (time).

$$V'_R = V_R - V_M \text{ and } t'_R = t_R - t_M$$

Source: Adapted from [1] 3.7.07

2.7.33 distribution constant (in chromatography), K_c

Concentration of a component in or on the *stationary phase* divided by the concentration of the component in the *mobile phase*.

Note 1: The concentration is calculated from the total amount of component present without regard to the existence of various forms (*e.g.* associated and dissociated forms).
Source: Adapted from [1] 3.9.01

2.7.34 hold-up volume (time) (in chromatography), V_M (t_M)

Volume of *mobile phase* (or the corresponding time) required to elute a component which is not retained by the *stationary phase*.

Note 1: The hold-up volume (time) includes any volumes contributed by the sample injector, the *detector*, and connectors.
Note 2: The hold-up time is related to the hold-up volume and *flow rate* by

$$t_M = V_M / F_c$$

Source: Adapted from [1] 3.7.03

2.7.35 linear retention index (in chromatography)
See *retention index*, Note 3.

2.7.36 relative retention (in chromatography), *r*

Adjusted retention volume (V'_R) (*adjusted retention time*, t'_R) or *retention factor* (k) of a component, i, divided by that of a reference compound, ref, obtained under identical conditions:

$$r = \frac{V'_{R,i}}{V'_{R,ref}} = \frac{t'_{R,i}}{t'_{R,ref}} = \frac{k_i}{k_{ref}}$$

Note 1: Depending on the relative position of the peak corresponding to the standard compound in the *chromatogram*, the value of r may be smaller, greater or identical to unity.
Source: Adapted from [1] 3.7.14.1

2.7.37 retardation factor in *planar chromatography*, R_f

Ratio of the distance travelled by the centre of the spot to the distance travelled by the mobile phase in a planar separation. Widely used in *thin-layer chromatography*.

Source [1] Adapted from 3.8.04

2.7.38 retention factor (in column chromatography), *k*
deprecated term: partition ratio
deprecated term: capacity ratio
deprecated term: capacity factor
deprecated term: mass distribution ratio
deprecated symbol: k'

Adjusted retention volume (*time*) divided by the *hold-up volume* (*time*).

$$k = \frac{V'_R}{V_M} = \frac{t'_R}{t_M}$$

Note 1: 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 terminologies (IUPAC, BS, ASTM) have always clearly identified the retention factor with the symbol k and not k'.
Note 2: 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 mobile phase.
Note 3: If the *distribution constant* 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. $k = \frac{1-R}{R}$, where R is the fraction of the sample component in the mobile phase (and therefore 1–R is the fraction in the stationary phase).
Source: Adapted from [1] 3.7.12

2.7.39 retention index (in chromatography), I_i
Kováts retention index

Number, obtained by interpolation (usually logarithmic), relating the *adjusted retention volume* (*adjusted retention time*) or the *retention factor* of a sample component (*i*) to the *adjusted retention volumes* (*adjusted retention times*) or *retention factors* of two standards eluted before and after the *peak* of the sample component.

Note 1: In Kováts retention index used in gas chromatography, n-alkanes serve as the standards and logarithmic interpolation is utilized:

$$I_i = 100\left(\frac{\log_{10}\frac{X_i}{X_z}}{\log_{10}\frac{X_{z+1}}{X_z}} + z\right),$$

where *X* refers to the *adjusted retention volume* or *adjusted retention time*, *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. Therefore $V'_{R,z} < V'_{R,i} < V'_{R,z+1}$.

Note 2: The Kováts retention index expresses the number of carbon atoms (multiplied by 100) of a hypothetical n-alkane, which would have an *adjusted retention volume* (*time*) identical to that of the peak of interest when analyzed under identical conditions.

Note 3: The *Kováts retention index* is always measured under isothermal conditions. In the case of *temperature-programmed gas chromatography*, a similar value can be calculated using numbers instead of their logarithms. 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:

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

where t^T_R refers to the total retention times 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.

Source: Adapted from [1] 3.7.15

2.7.40 separation factor (in chromatography), α
deprecated: selectivity

Ratio of the *adjusted retention volumes* (V'_R), *adjusted retention times* (t'_R) or *retention factors* (*k*) of two adjacent peaks expressed as a number greater than one:

$$r = \frac{V'_{R,2}}{V'_{R,1}} = \frac{t'_{R,2}}{t'_{R,1}} = \frac{k_2}{k_1}$$

where $V'_{R2} > V'_{R1}$

Source: Adapted from [1] 3.7.14.2

2.7.41 total retention volume (time) (in chromatography), V_R (t_R)

Volume of *mobile phase* entering a *column* between sample injection and the emergence of the peak maximum of the sample component of interest, or the corresponding time.

Note 1: Total retention volume (time) includes the *hold-up volume* (*time*).

Note 2: The total retention time is related to the total retention volume and *flow rate* by $t_R = \frac{V_R}{F_c}$

2.8 Terms expressing the efficiency of separation

2.8.1 effective plate height, H_{eff}

height equivalent to one effective plate
deprecated term: height equivalent to one effective theoretical plate
deprecated symbol: H

Column length divided by *effective plate number*:

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

Source: [1] (with minor change) 3.10.06

2.8.2 effective plate number, N_{eff}

number of effective plates
deprecated term: number of effective theoretical plates
deprecated symbol: N

Number indicative of column performance, calculated from the ratio of measures of adjusted retention and *peak width*:

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

$$N_{eff} = 16\left(\frac{V'_R}{w_b}\right)^2 = 16\left(\frac{t'_R}{w_b}\right)^2$$

$$N_{eff} \approx 5.545\left(\frac{V'_R}{w_h}\right)^2 \approx 5.545\left(\frac{t'_R}{w_h}\right)^2$$

Note 1: V'_R: *adjusted retention volume*; T'_R: *total retention time*; σ: standard deviation of the Gaussian peak; w_b: *peak width at base*; w_h: *peak width at half height*; $8 \ln 2 \approx 5.545$.

Note 2: These expressions assume a Gaussian (symmetrical) peak.

Note 3: Units for the quantities being divided must be consistent so that their ratio is dimensionless: *i.e.* if the numerator is a time, then peak width must also be expressed in terms of time.

Note 4: The *plate number* is related to the effective plate number and *retention factor* (k) $N = N_{eff}\left(\frac{k+1}{k}\right)^2$

Source: [1] (with minor change) 3.10.04

2.8.3 peak resolution (in chromatography), R_s

The separation of two *peaks* expressed as the difference in the *retention times* ($t_{R2} > t_{R1}$) divided by their average *peak width at base* with units of time.

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

Note: 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:

$$R_s \approx \frac{t_{R2} - t_{R1}}{w_{b2}}$$

Source: Adapted from [1] 3.10.1

2.8.4 plate height, H

height equivalent to one theoretical plate (HETP)
deprecated symbol: h

Column length divided by *plate number*.

$$H = L/N$$

Source: [1] (with minor change) 3.10.06

2.8.5 plate number, N

number of theoretical plates
theoretical plate number
deprecated symbol: n

Number indicative of column performance, calculated from the ratio of measures of retention and *peak width*:

$$N = \left(\frac{V_R}{\sigma}\right)^2 = \left(\frac{t_R}{\sigma}\right)^2$$

$$N = 16\left(\frac{V_R}{w_b}\right)^2 = 16\left(\frac{t_R}{w_b}\right)^2$$

$$N \approx 5.545\left(\frac{V_R}{w_h}\right)^2 \approx 5.545\left(\frac{t_R}{w_h}\right)^2$$

Note 1: V_R: *total retention volume*; t_R: *total retention time*; σ: standard deviation of the Gaussian peak; w_b: *peak width at base*; w_h: *peak width at half height*; $8 \ln 2 \approx 5.545$.
Note 2: These expressions assume a Gaussian (symmetrical) peak.
Note 3: Units for the quantities being divided 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.
Source: [1] (with minor change) 3.10.03

2.8.6 reduced plate height, *h*

In *liquid chromatography plate height* divided by average particle diameter.

$$h = H/d_p$$

Note 1: For *open-tubular columns h* is the plate height divided by the *column diameter*. $h = H/d_c$
Source: [1] (with minor change) 3.10.07

2.8.7 separation number, *SN*

Number of peaks which can be resolved in a gas *chromatogram* between the peaks of two consecutive n-alkanes with z and $(z+1)$ carbon atoms in their molecules:

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

Note 1: The separation number is calculated from *retention times* and *peak widths at half height* of the peaks
Note 2: In the German literature, the symbol *TZ* ('Trennzahl') is commonly used as the symbol for the separation number.
Note 3: As the separation number depends on the n-alkanes used for the calculation, they always must be specified with any given *SN* value.
Source: Adapted from [1] 3.10.02

2.9 Terms related to detection

Classification of Detectors

2.9.1 detector (in chromatography)

Sensor that responds to components in the *effluent*.

Example: ultraviolet spectrometer, mass spectrometer, flame ionization detector

2.9.2 selective detector (in chromatography)

Detector which responds to a related group of components in the *effluent*.

Example: Spectroscopic detector that monitors all compounds with an absorbance at a particular wavelength.
Source: [1] (with minor change) 4.1.03.2

2.9.3 specific detector (in chromatography)

Detector which responds to a single sample component or to a limited number of components having similar chemical characteristics.

Example: An element specific detector responds to nitrogen, sulfur, and phosphorous in a molecule. The more of these elements in the molecule, the greater the detector response.

Source: [1] (with minor change) 4.1.03.3

2.9.4 universal detector (in chromatography)

Detector which responds to every component in the *effluent* except the *mobile phase*.

Source: [1] (with minor change) 4.1.03.1

2.10 Special terminology used in different modes of chromatography

2.10.1 Supercritical fluid chromatography and extraction

Supercritical fluid chromatography (SFC) has led to the use in the literature of new terms whose meanings have generally been adopted by workers in the field. Supercritical fluids have also been used for the extraction of samples. Frequently, equipment and operating conditions similar to those used in SFC have been employed and many of the terms used in SFC are also applicable in this field.

Basic Definitions

2.10.1.1 critical point

The characteristic temperature (T_c) and pressure (p_c) above which a gas cannot be liquefied.

Source: [10]

2.10.1.2 critical pressure, p_c

Minimum pressure which would suffice to liquefy a substance at its *critical temperature*.

Note 1: Above the critical pressure, increasing the temperature will not cause a fluid to vaporise to give a two-phase system.

Source: [10]

2.10.1.3 critical temperature, T_c

Maximum temperature at which a gas can be converted into a liquid by an increase in pressure.

Source: [10]

2.10.1.4 supercritical fluid

State of a compound, mixture, or element above its *critical pressure* (p_c) and *critical temperature* (T_c).

Source: [10] (with minor change)

Terms Related to the Chromatographic Process

2.10.1.5 isobaric separation

Chromatographic separation carried out using constant inlet and outlet pressure conditions.
Source: [10]

2.10.1.6 isopycnic separation
deprecated term: isoconfertic separation

Chromatographic separations carried out using constant density conditions.

Note 1: Temperature and pressure may be altered during the run.
Source: [10]

2.10.2 Counter-current chromatography (CCC)

Counter-current chromatography uses many terms already defined for chromatography

2.10.2.1 column volume (in counter-current chromatography)

The volume of the active element of the column or coil containing the *stationary phase* and the *mobile phase*.

2.10.2.2 distribution ratio (in counter-current chromatography), K_C
deprecated term: distribution coefficient
deprecated term: distribution constant

Concentration of a solute in the liquid *stationary phase*, regardless of its chemical form, divided by its total concentration (all forms) in the *mobile phase*.

Note 1: The distribution ratio varies with experimental conditions, *e.g.* pH, presence of complexing agents. It should not be confused with *distribution constant* (or partition coefficient, *P*, a term not recommended but still used), which applies to a particular chemical species and is by definition invariable.
Note 2: The distribution ratio is a measured value and does not necessarily imply that distribution equilibrium between the liquid phases has been achieved.
Note 3: The distribution ratio of a solute is directly proportional to its CCC *retention time* or *retention volume*.

2.10.2.3 elution mode (in counter-current chromatography)

Method of introduction of the *mobile phase* (upper or lower) into the rotating coil (from the head or the tail). Of four combinations, only two elution modes should be used in *high-speed counter-current chromatography*, *i.e.* either the lower phase from the head toward the tail or the upper phase from the tail toward the head.

Source: Adapted from [11]

2.10.2.4 high-speed counter-current chromatography (HSCCC)

Counter-current chromatography system using a multilayer coil separation column, which undergoes a type-J synchronous planetary motion (see Fig. 3).

Note 1: The method covers analytical (microgram) to preparative (10 g) scale separations usually in a few hours at a high partition efficiency up to a few thousand theoretical plates (see *number of theoretical plates*).

Source: Adapted from [11]

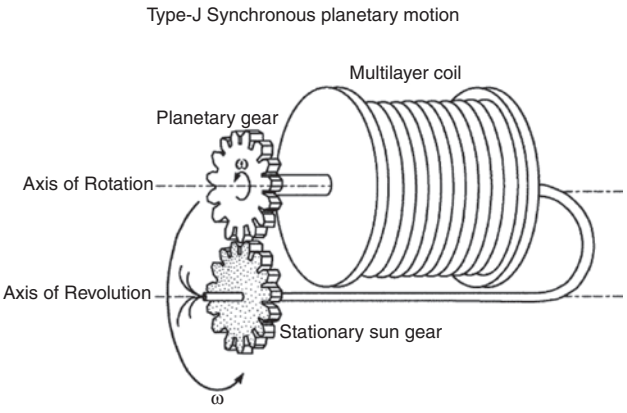

Fig. 3: Type-J planetary motion of a multilayer coil separation column. The column holder rotates about its own axis and revolves around the centrifuge axis at the same angular velocity (ω) in the same direction. This planetary motion prevents twisting of the bundle of flow tubes, allowing continuous elution through a rotating column without risk of leakage and contamination.

2.10.2.5 mobile phase (in counter-current chromatography)

One of the two solvent phases, either upper or lower, which elutes through the *column*.

Source: Adapted from [11]

2.10.2.6 mobile-phase volume (in counter-current chromatography), V_M

The volume of the liquid *mobile phase* contained in the *column* in *counter-current chromatography*

$$V_M = V_C - V_S$$

where V_C is the total volume of the *column* and V_S is the volume of the liquid *stationary phase* in the *column*.

2.10.2.7 multilayer coil (in counter-current chromatography)

A separation column used for *high-speed counter-current chromatography*.

Note 1: The multilayer coil is prepared from a long piece of polytetrafluoroethylene (PTFE) tubing by coaxially winding it around a spool-shaped holder, making multiple coiled layers.

Note 2: Usually, three different inner diameter sizes of tubing are used: ca. 1 mm for analytical, 1.6 mm for semipreparative, and 2.6 mm for preparative separations. It provides efficient mixing of the two phases and a high retention of the stationary phase under a high *flow rate* of the mobile phase.

Source: Adapted from [11]

2.10.2.8 phase volume ratio (in counter-current chromatography), β_V

The ratio of the volume of the *mobile phase* to that of the *stationary phase* in a *column* in *counter-current chromatography*

Note 1: The stationary phase volume retention ratio (S_f) has been defined as the ratio of the volume of *stationary phase* to total column/coil volume, $S_f = V_s/V_c$

Note 2: It is recommended that phase ratio in *counter-current chromatography* be given the subscript V (*i.e.* $\beta_V = V_M/V_S$) to emphasize phase volume ratio.

Note 3: β in *counter-current chromatography* has been defined as the ratio of planetary over main rotor radius, $\beta = r/R$

2.10.2.9 pH-zone-refining counter-current chromatography

Counter-current chromatography that produces a train of fused rectangular solute peaks called pH-zones, each with its specific pH. Each zone contains highly concentrated solute, often near saturation level, and is separated from the neighbouring zone by a narrow strip called a mixing zone, which contains a mixture of impurities.

Source: Adapted from [11]

2.10.2.10 retainer and eluter (in counter-current chromatography)

Most important reagents in *pH-zone-refining counter-current chromatography*. A retainer is added to the stationary organic phase to retain the analytes and the eluter added to the mobile aqueous phase to elute the analytes.

Note 1: Trifluoroacetic acid and triethylamine are typical retainers for the separation of acidic and basic compounds, respectively, where the *stationary phase* typically is the organic phase. The retainer determines the analyte concentration in the *stationary phase*. NH_4OH and HCl are commonly used as the eluter for the separation of acidic and basic compounds, respectively. The eluter always stays in the aqueous mobile phase to serve as a counter ion. *pH-zone-refining CCC* can also be performed using the organic phase as the *mobile phase* (normal displacement mode). In this case, the retainer becomes the eluter to elute the analytes, and the eluter becomes the retainer to retain the analytes in the aqueous *stationary phase*.

Source: Adapted from [11]

2.10.2.11 retention volume of the stationary phase (in counter-current chromatography), V_S

Volume of the *stationary phase*, which stays permanently within the *column* in *counter-current chromatography*.

Note 1: V_S is one of the most important parameters that determines the resolution.

Source: Adapted from [11]

2.10.2.12 type-J coil planet centrifuge

Centrifuge that provides *type-J synchronous planetary motion* to the column holder, the axis of which is parallel to the centrifuge axis (see Fig. 3).

Source: Adapted from [11]

2.10.2.13 type-J synchronous planetary motion

Unique type of planetary motion (so named because the flow tubes form a letter J figure, as shown in Fig. 3) of the column holder, which rotates around its own axis once during one revolution around the central axis of the centrifuge in the same direction.

Note 1: The system prevents the flow tubes from twisting during column rotation, and produces an excellent mixing and high retention of the *stationary phase* in a multilayer coil separation column.

Source: Adapted from [11]

2.10.2.14 zone pH (in counter-current chromatography), pH_{zone}

pH of the solute zone in pH-*zone-refining counter-current chromatography.*

$pH_{zone} = pKa + \log_{10}[(K_{DS}/K_S) - 1]$, where K_{DS} is the partition ratio of solute S (an indicator for solute hydrophobicity) and K_S, the dissociation constant of the solute.

Note 1: According to theoretical analysis two solutes that differ their zone pH values by 0.2 or greater can be resolved in pH-zone-refining CCC.

Source: Adapted from [11]

2.10.3 Ion-exchange chromatography

Basic Definitions

2.10.3.1 anion exchange

Exchange of anions between a solution and an anion exchanger.

Source: [1] (with minor change) 5.1.10

2.10.3.2 cation exchange

Exchange of cations between a solution and a cation exchanger.

Source: [1] (with minor change) 5.1.09

2.10.3.3 co-ions

Mobile ionic species in an *ion exchanger* with a charge of the same sign as the fixed ions.

Source: [1] (with minor change) 5.1.08

2.10.3.4 counter-ions (in ion-exchange chromatography)

Mobile exchangeable ions in *ion-exchange chromatography,* with a charge of opposite sign to the fixed ions.

Source: [1] (with minor change) 5.1.02

2.10.3.5 fixed ions

Ion exchanger in which the non-exchangeable ions have a charge opposite to that of the counter-ions.

Source: [1] 5.1.03

2.10.3.6 ion-exchange isotherm

Ion-exchange chromatography in which the concentration of a counter-ion in the *ion exchanger* is expressed as a function of its concentration in the external solution under specified conditions and at constant temperature.

Source: [1] (with minor change) 5.1.04

2.10.3.7 ionogenic groups

Fixed groupings in an *ion exchanger* that are either ionized or capable of dissociation into fixed ions and mobile counter-ions.

Source: [1] (with minor change) 5.1.07

2.10.3.8 sorption (in ion-exchange chromatography)

Uptake of electrolytes or non-electrolytes by an *ion exchanger* through mechanisms other than pure ion exchange.

Source: [1] (with minor change) 5.1.05

2.10.3.9 sorption isotherm (in ion-exchange chromatography)

Concentration of a sorbed species in an *ion exchanger*, expressed as a function of its concentration in the external solution under specified conditions and at constant temperature.

Source: [1] (with minor change) 5.1.06

The Chromatographic Medium in Ion Exchange Chromatography

2.10.3.10 ion exchanger

Solid inorganic or organic substance containing exchangeable ions.

Note 1: It is recognized that there are also 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.

Note 2: A monofunctional ion exchanger contains only one type of ionogenic group, a bifunctional ion exchanger two types, and a polyfunctional ion exchanger more than one type. In a macroporous ion exchanger, the pores are large compared to atomic dimensions.

Source: Adapted from [1] 5.3.01

2.10.3.11 salt form of an ion exchanger

Ionic form of an *ion exchanger* in which the counter-ions are neither hydrogen nor hydroxide ions.

Note 1: 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.

Note 2: 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.

Source: [1] (with minor change) 5.3.01.6

2.10.3.12 redox ion exchanger

Conventional *ion exchanger* in which a reversible redox couple has been introduced as counter-ions either by sorption or complex formation.

Note 1: Redox ion exchangers closely resemble redox polymers in their behaviour.

Source: [1] (with minor change) 5.3.01.8

2.10.4 Enantioselective (chiral) chromatography

2.10.4.1 chiral additive

Chiral selector that has been added as a component of a *mobile phase* or electrophoretic medium.

Source: [5]

2.10.4.2 chiral bonded stationary phase

Chiral stationary phase in which a *chiral selector* has been chemically bonded to the surface of a solid support or column wall.

Source: Adapted from [5]

2.10.4.3 chiral mobile phase

Mobile phase containing a *chiral selector*.

Source: [5]

2.10.4.4 chiral selector

Chiral component of the separation system capable of interacting enantioselectively with the enantiomers to be separated.

Source: [5]

2.10.4.5 chiral stationary phase

Stationary phase which incorporates a *chiral selector*.

Note 1: If not a constituent of the stationary phase as a whole, the chiral selector can be chemically bonded to (*chiral bonded stationary phase*) or immobilized onto the surface of (*chiral coated stationary phase*) a solid support or column wall, or simply dissolved in the liquid stationary phase.

Source: Adapted from [5]

2.10.5 Two-dimensional chromatography

2.10.5.1 comprehensive two-dimensional chromatography
comprehensive chromatography

Type of *two-dimensional chromatography* in which all the *effluent* from the first separation step is transferred in fractions to a second separation step.

Note 1: The multiplier (×) is used to denote the two separation steps, *e.g.* $GC \times GC$ or $LC \times SFC$

2.10.5.2 heart-cut separations

Type of *two-dimensional chromatography* in which selected fractions from the first separation step are transferred to a second separation step.

2.10.5.3 modulation frequency (in comprehensive chromatography), f_M

Number of modulations in a given time interval.

Source: [12]

2.10.5.4 modulation number (in comprehensive chromatography), n_M

The number of modulations for a given first-dimension peak.

Source: [12]

2.10.5.5 modulation period (in comprehensive chromatography), P_M
modulation time

Duration of a complete cycle of modulation in a system for *comprehensive two-dimensional chromatography*

Note 1: Modulation period is the length of a second dimension chromatogram, *i.e.* the time between two successive injections into the second column.

Source: [12]

2.10.5.6 modulator (in comprehensive chromatography)

Interface device between the two *columns* in a system for *comprehensive two-dimensional chromatography* that accumulates or samples narrow bands from the eluate of the first column for fast re-injection into the second column.

Source: [12]

2.10.5.7 multi-dimensional chromatography

Chromatography in which parts or all of the separated sample components are subjected to additional separation steps.

Note 1: Multi-dimensional chromatography can be done, for example, by conducting a particular fraction eluting from the column into other columns (system) having different separation characteristics.

Source: [1] (with minor change) 1.6.06

2.10.5.8 two-dimensional chromatography

Multi-dimensional chromatography with one additional separation step.

Note 1: 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*.

Source: [1] (with minor change) 1.6.06

2.10.5.9 wrap-around (in comprehensive chromatography)

Occurrence of second dimension *peaks* in subsequent elution sequences, caused by second-dimension retention times that exceed the *modulation time* in *comprehensive two-dimensional chromatography*.

Source: [12]

2.10.6 Exclusion chromatography

Besides the terms and definitions used in general in chromatography, special terms exist in *exclusion chromatography*. In addition, due to the different nature of the chromatographic separation, some of the general chromatographic terms have a different meaning here. For further explanation of some of the terms, see Fig. 4.

Below, only the chromatography terms are listed. For a discussion of the molecular weight terms calculated from the chromatographic data, see specialized vocabularies.

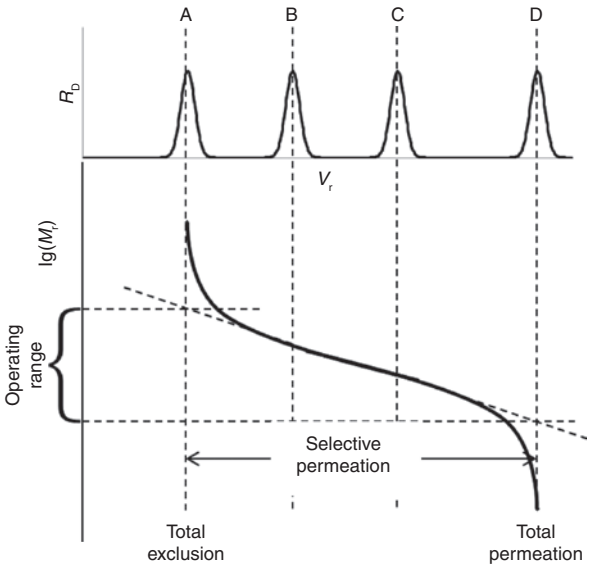

Fig. 4: Retention characteristics in *exclusion chromatography*. A standard sample is analyzed (top); subsequently, the logarithms of the corresponding relative molecular mass ('molecular weights'), lg(M_r), are plotted against the *retention volumes,* V_R. One can also plot the logarithm of the relative molecular mass ('molecular weight'), lg(Mr), against the retention time, t_R. Peak A corresponds to a non-retained sample component the molecules of which are larger than the largest pores in the stationary phase particles (total exclusion); peak D corresponds to a sample component the molecules of which are smaller than the smallest pores in the particles of the stationary phase (total penetration).

The Column

2.10.6.1 interparticle volume of the column (in size-exclusion chromatography), V_0
interstitial volume

Volume of the *mobile phase* in the interstices between particles that comprise the *stationary phase*.

Note 1: 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; see *total mobile-phase volume in column*). The reason for this is that in 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 *interparticle volume of the column* (*in chromatography*)) and the retention volume of a non-retained compound are practically equal.

Source: [1] (with minor change) 3.2.11

2.10.6.2 intraparticle volume of the column (in size-exclusion chromatography), V_i
intrastitial volume of the column
stationary mobile-phase volume

Volume of the *mobile phase* within the pores of the particles in the stationary phase.

Note 1: The *retention time* equivalent to V_i is t_i

$$t_i = \frac{V_i}{F_c}$$

Source: [1] (with minor change) 6.1.02

2.10.6.3 total mobile-phase volume in column (in size-exclusion chromatography), V_t

The sum of the *interparticle volume of the column* and *intraparticle volume of the column*: $V_t + V_0 + V_i$

Note 1: In the definition of V_t the *extra-column volume* of the system (V_{ext}) is neglected. If it is not negligible, it must also be added:

$$V_t = V_0 + V_i + V_{ext}$$

Source: [1] (with minor change) 6.1.03

Retention Parameters

2.10.6.4 adjusted retention volume (time) (in size-exclusion chromatography), V'_R (t'_R)

Total retention volume minus the *retention volume* of an unretained compound:

$$V' = V_R - V_0$$

The corresponding retention time is t'_R:

$$t'_R = t_R - t_0 = \frac{V'_R}{F_c} = \frac{V_R - V_0}{F_c}$$

Source: [1] (with minor change) 6.2.03

2.10.6.5 distribution constant (in size-exclusion chromatography), K_0

Fraction of the intraparticle volume (the volume of the pores) available to the molecules of a particular sample component for diffusion:

$$K_0 = \frac{V_R - V_0}{V_i}$$

Note 1: The value of K_0 varies between zero and unity. For an unretained compound, $V_R = V_0$ and thus, $K_0 = 0$. On the other hand, for a compound the molecules of which are smaller than the smallest pores, $V_R = V_t$ and thus, $K_0 = 1$.

Note 2: In *exclusion chromatography*, K_0 is related to the retention volume of a sample component and the *inter-* and *intraparticle volumes of the column* (V_0 and V_i, respectively) in a manner analogous to the relationship in general *liquid chromatography*:

$$V_R = V_0 + K_0 V_i$$

Source: [1] (with minor change) 6.2.06

2.10.6.6 retention factor (in size-exclusion chromatography), k_e

The ratio of the *adjusted retention volume* (*time*) and the *retention volume* (*time*) of an unretained compound:

$$k_e = \frac{V_R - V_0}{V_0} = \frac{t_R - t_0}{t_0}$$

Source: [1] (with minor change) 6.2.05

2.10.6.7 retention volume (time) of an unretained compound (in size-exclusion chromatography), V_0 (t_0)

Retention volume of a sample component the molecules of which are larger than the largest pores of the particles of the stationary phase.

Note 1: These molecules will be eluted first from the column. The corresponding retention time is t_0:

$$t_0 = \frac{V_0}{F_c}$$

Note 2: Ignoring any extra-column volume, V_0 is equal to the interparticle volume of the column.
Source: [1] (with minor change) 6.2.01

2.10.6.8 retention volume (time) (in size-exclusion chromatography), V_R (t_R)

Retention volume (time) of a sample component, the molecules of which are smaller than the largest pores of the particles of the stationary phase but larger than the smallest pores.
Note 1: The corresponding retention time is t_R:

$$t_R = \frac{V_R}{F_c}$$

Source: [1] (with minor change) 6.2.02

2.10.6.9 total mobile phase volume (time) (in size-exclusion chromatography), V_t (t_t)

Retention volume (*time*) of a sample component, the molecules of which are smaller than the smallest pores of the particles of the *stationary phase*. The corresponding retention time is t_t:

$$t_t = \frac{V_t}{F_c}$$

Source: [1] (with minor change) 6.2.04

Efficiency Terms

2.10.6.10 relative molar-mass exclusion limit (in polymers)
molecular-weight exclusion limit

Maximum value of the relative molar mass of molecules or particles in a specific polymer solvent system that can enter into the pores of the porous non-adsorbing material used in *size-exclusion chromatography*.

Note 1: For particles with relative molar mass greater than the exclusion limit the separation effect of the size-exclusion chromatography vanishes.
Source: [13]

2.10.6.11 specific resolution (in size-exclusion chromatography), R_{sp}

Peak resolution also considering the molecular masses (M_1, M_2) of the two test compounds:

$$R_{sp} = \left[\frac{V_{R1} - V_{R2}}{\frac{w_{b1} + w_{b2}}{2}}\right] \cdot \left[\frac{1}{\log_{10}\frac{M_2}{M_1}}\right]$$

Note 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 2: In some areas, the symbol R_s is used for the specific resolution. Due to the possibility of confusing it with the general resolution term (see *peak resolution*), the symbol R_{sp} is suggested here.

Source: [1] (with minor change) 6.3.02

2.10.6.12 spreading function

In *size-exclusion chromatography*, normalized response of a detector expressed as a function of *elution* volume by an instantaneous injection of a uniform sample.

Source: [13]

2.10.6.13 universal calibration (in size-exclusion chromatography)

Calibration in *size-exclusion chromatography* based on the principle that the *retention volume* of a molecular or particulate species is a single-valued function of an appropriate size parameter of the molecule or particle, irrespective of its chemical nature and structure.

Note 1: The product of the intrinsic viscosity and molar mass, $\eta_{rmi} \times M$, has been widely used as the size parameter.

Source: [13]

3 Electromigration techniques

3.1 capillary affinity electrophoresis (CAE)

affinity capillary electrophoresis (ACE)

Capillary electrophoresis in which the background electrolyte contains substances capable of specific, often biospecific, interactions with the analytes.

Source: [14] (with minor change)

3.2 capillary electro-chromatography (CEC)

Liquid chromatography where the movement of the mobile phase through a capillary, filled, packed, or coated with a *stationary phase*, is achieved by electroosmotic flow (which may be assisted by pressure).

Note 1: The *retention time* is determined by a combination of electrophoretic migration and chromatographic retention.

Source: [14] (with minor change)

3.3 capillary electrophoresis (CE)

capillary zone electrophoresis (CZE)

Separation method based solely on the differences in the *electrophoretic mobilities* of charged species in capillaries.

Note 1: The background electrolyte may be an aqueous or non-aqueous solution.
Note 2: The electrolytes can contain additives, which can interact with the charged species and alter their *electrophoretic mobilities*. See *capillary affinity electrophoresis*.
Note 3: The use of the term capillary electrophoresis (CE) as a collective term for all capillary electromigration techniques is not recommended, as some of these techniques involve separation mechanisms other than electrophoresis.
Source: [14] (with minor change)

3.4 capillary gel electrophoresis (CGE)

Capillary sieving electrophoresis in which the capillary is filled with a gel.

Source: [14] (with minor change)

3.5 capillary isoelectric focusing (CIEF)

Electrophoretic technique for the separation of analytes according to their isoelectric points by an electric field along a pH gradient formed in a capillary.

Source: [14] (with minor change)

3.6 capillary isotachophoresis (CITP)

Electrophoretic separation technique in a discontinuous buffer system in which analytes migrate according to their *electrophoretic mobilities*, forming a chain of adjacent zones moving with equal speed (velocity) between two solutions, leading and terminating electrolytes, bracketing the mobility range of the analytes.

Source: [14] (with minor change)

3.7 capillary sieving electrophoresis (CSE)

Electrophoretic separation technique, which takes place in a capillary containing a medium in the background electrolyte that retains molecules and particles based on their size and shape.

Note 1: The medium may be an entangled polymer network.
Source: Adapted from [14]

3.8 effective length of the capillary, L_{eff}

Length of the capillary from the sample introduction point to the detection point.

Source: Adapted from [13]

3.9 electric field strength, E
electric field

Force acting on a charge divided by the charge.

Note 1: In *capillary electromigration*, this is taken as the axial component of the field, that is, the difference between the applied electric potential at the sample introduction end of the capillary and that at the detection end, divided by the total length of the capillary, L_{tot}. There is also a radial electric field arising from the electrical double layer, which is involved in *electroosmosis*. The total length of the capillary is typically greater than the *effective length of the capillary*, L_{eff}. See *migration time*.

Source: [14] (with minor change)

3.10 electrokinetic chromatography (EKC)

electrokinetic capillary chromatography (ECC)

Separation method based on a combination of electrophoresis and interactions of the analytes with additives (*e.g.* surfactants), which form a dispersed phase moving at a different speed (velocity). In order to achieve separation, either the analytes or this secondary phase should be charged.

Note 1: This is not totally compatible with the definition of *chromatography*.

Source: [14] (with minor change)

3.11 electrokinetic injection

Injection of the analytical sample by applying a potential across the sample and capillary and using the EOF to draw aliquot into the end of the capillary tube. The sample is then replaced by the running electrolyte for the separation to take place.

3.12 electrokinetic potential, ζ

zeta potential

ζ-potential

Potential difference across the mobile part of the interfacial double-layer, that is responsible for electrokinetic phenomena.

Note 1: ζ is positive if the potential increases from the bulk of the liquid phase towards the interface.

Note 2: In calculating the electrokinetic potential from electrokinetic phenomena, it is often assumed that the liquid adhering to the solid wall and the mobile liquid are separated by a sharp shear plane. As long as there is no reliable information on the values of the permittivity and the viscosity in the electrical double layer close to the interface, the calculation of the electrokinetic potential from electrokinetic experiments is open to criticism. It is therefore essential to indicate in all cases which equations have been used in the calculation of ζ. It can be shown, however, that for the same assumptions about the permittivity and viscosity all electrokinetic phenomena must give the same value for the electrokinetic potential.

Note 3: If the conditions referred to are followed, the electrokinetic potential can be related to the *electroosmotic mobility*, μ_{eo}, by the Smoluchowski equation:

$$\mu_{eo} = -\frac{\varepsilon_r \cdot \varepsilon_0 \cdot \zeta}{\eta}$$

where ε_r is the relative permittivity of the solution (usually taken as that of the pure solvent), ε_0 is the permittivity of vacuum, and η is the dynamic viscosity of the solution, where the ratio of permittivity to viscosity is assumed to be independent of the electric field of the double layer.

Source: [14] (with minor change)

3.13 electroosmosis
electro-osmosis
deprecated term: electrosmosis
obsolete term: electroendosmosis

Motion of a liquid through a capillary (or membrane or plug) because of the application of an electric field across the capillary (or membrane or plug).

Source: [14] (with minor change)

3.14 electroosmotic hold-up time (in capillary electromigration), t_{eo}

Time required for a liquid in a capillary to move, due to *electroosmosis*, through the *effective length of the capillary*, L_{eff}.

Note 1: t_{eo} is usually measured as the *migration time* of a neutral compound, which is assumed to have an *electroosmotic mobility* that is negligible compared to that of the analyte.
Source: [14] (with minor change)

3.15 electroosmotic mobility (in capillary electromigration), u or μ_{eo}

Electroosmotic velocity divided by electric field strength.

Source: [14] (with minor change)

3.16 electroosmotic velocity, v_{eo}

Effective length of the capillary divided by the *electroosmotic hold-up time*.

$$v_{eo} = L_{eff}/t_{eo}$$

Note 1: In *capillary electromigration*, this velocity is positive, by convention, in the direction from the sample introduction end of the capillary to the detection end, otherwise negative.
See *electroosmosis*
Source: Adapted from [14]

3.17 electroosmotic volume flow rate
electroosmotic volume flow

Volume *flow rate* divided by electrical field strength through the whole plug or capillary.

Source: [14] (with minor change)

3.18 electrophoretic mobility, μ

Observed *velocity of migration* ($\boldsymbol{v}$) of a component divided by *electric field strength* (E) in a given medium.

Note 1: Mobilities are sometimes expressed with a negative sign, because migration of the solutes or particles generally occurs in a direction opposite to the electrophoretic field (which is taken as a reference for that direction).

Note 2: In a solid support medium, only apparent values can be measured.
Source: [14] (with minor change)

3.19 electrophoretic velocity, ν_{ep}

velocity of migration (in electrophoresis)

Velocity of a charged analyte under the influence of an electric field relative to the background electrolyte equal to the distance of migration divided by time of migration.

Source: [13] with changes

3.20 electrostacking

Methods that use differences in migration behaviour in spatially defined regions of the capillary of different field strengths (conductivities).

Note 1: Electrostacking methods can be used to concentrate dilute samples.

3.21 hydrodynamic injection

Introduction of the analytical sample into a capillary by a pressure difference between the ends of the capillary.

3.22 immunoelectrophoresis

Biochemical methods for separation and characterization of proteins based on electrophoresis and reaction with antibodies.

3.23 leading electrolyte

See *sample introduction in isotachophoresis separations*

3.24 mass distribution ratio (in micellar electrokinetic chromatography), k_{MEKC}

$$k_{MEKC} = \frac{n_{mc}}{n_{aq}} = K\frac{V_{mc}}{V_{aq}}$$

where n_{mc} and n_{aq} are the chemical amounts of the analyte in the micellar and aqueous phases, respectively. K is the *distribution constant* and V_{mc} and V_{aq} are the corresponding volumes of the phases.

Note 1: In the case of an electrically neutral analyte, k_{MEKC} can be calculated directly from the migration time of the analyte, t_m, the *migration time of the micelles*, t_{mc}, and the *electroosmotic hold-up time*, t_{eo}:

$$k_{MEKC} = \frac{t_m - t_{eo}}{t_{eo}}\left(1 - \frac{t_m}{t_{mc}}\right)$$

Note 2: k_{MEKC} should not be confused with the *retention factor* (in column chromatography) k. However, k_{MEKC} is analogous to the *mass distribution ratio* (in chromatography).

Note 3: The definition is analogous to mass distribution ratio (in *microemulsion electrokinetic chromatography*), k_{MEEKC} by replacing terms for microemulsions with corresponding terms for micelles.

Source: [14]

3.25 mass distribution ratio (in microemulsion electrokinetic chromatography), k_{MEEKC}

$$k_{MEEKC} = \frac{n_{mec}}{n_{aq}} = K\frac{V_{mc}}{V_{aq}}$$

where n_{mec} and n_{aq} are the chemical amounts of the analyte in the microemulsion and aqueous phases, respectively. K is the *distribution constant* and V_{mec} and V_{aq} are the corresponding volumes of the phases.

Note 1: The definition is analogous to mass distribution ratio (in *micellar electrokinetic chromatography*), $k_{MEKC,}$ replacing terms for micelles with corresponding terms for microemulsion.

Source: [14] (with minor change)

3.26 micellar electrokinetic chromatography (MEKC)

micellar electrokinetic capillary chromatography (MECC)

Electrokinetic chromatography, in which the secondary phase is a micellar dispersed phase in the capillary.

Source: [14] (with minor change)

3.27 microemulsion electrokinetic chromatography (MEEKC)

microemulsion electrokinetic capillary chromatography (MEECC)

Electrokinetic chromatography, where a microemulsion is employed as the dispersed phase.

Source: [14] (with minor change)

3.28 migration time of micelles (in micellar electrokinetic chromatography), t_{mc}

Measured *migration time* of a compound that is completely retained in the micellar phase.

Source: [14] (with minor change)

3.29 migration time (in electrophoresis), t_m

Time required for the analyte to move through *the effective length of the capillary*, that is, from sample introduction point to detection point.

Source: [14] (with minor change)

3.30 non-aqueous capillary electrophoresis (NACE)

Capillary electrophoresis, which uses water-free organic solutions and modifiers to produce ions in solution.

Note 1: Typical organic solvents are methanol, ethanol, propanol, and acetonitrile.

Note 2: Typical modifiers are acetic acid, sodium acetate, ammonium acetate, ammonium chloride and ammonium hydroxide (non aqueous) [14, 15]

3.31 sample introduction in isotachophoresis separations

Sample is introduced between a zone of fast *leading electrolyte* and a zone of slow *terminating electrolyte*.

Note: Usually, the leading and terminating electrolytes have a common counter-ion, but the co-ions are different.

3.32 terminating electrolyte

See *sample introduction in isotachophoresis separations*

3.33 total velocity of the analyte (in capillary electrophoresis), $\boldsymbol{\nu}_{tot}$

Sum of the *electrophoretic velocity* $\boldsymbol{\nu}_{ep}$ of an ion and the *electroosmotic velocity* $\boldsymbol{\nu}_{eo}$.

$$\boldsymbol{\nu}_{tot} = \boldsymbol{\nu}_{ep} + \boldsymbol{\nu}_{eo}$$

Note 1: $|\boldsymbol{\nu}_{tot}|$ can be measured experimentally as the *effective length of the capillary* divided by the *migration time* (L_{eff}/t_m).

Note 2: Depending on the signs and relative magnitudes of these velocities, the total velocity of an analyte can have either the same or the opposite direction to the electroosmotic velocity.

Note 3: The total speed is the speed of the ion measured as a displacement relative to the capillary wall divided by time.

Source: [14] (with minor change)

4 Field-flow fractionation and related techniques

4.1 asymmetrical flow field-flow fractionation (AsFlFFF, AF4)

Flow field-flow fractionation in which only one channel wall is permeable, meaning there is one channel inlet for the flow during the run and some of the channel flow is directed through the accumulation wall.

Note 1: In a uniform width channel this would result in the channel flow decreasing dramatically along the channel length. In order to minimize this, a trapezoid shape of the separation channel has been developed for AsFlFFF. In general, the channel geometry significantly affects the separation efficiency in AF4.

Note 2: Three different channel designs were evaluated using polystyrene latex nanoparticles. The channel breadth was held constant for one (rectangular) profile, and was reduced either linearly (trapezoid profile) or exponentially (exponential profile) along the length for the other two channels. Compared with a rectangular channel, trapezoid and exponential profiles provided better performance of AsFlFFF and allowed band broadening to be reduced.

Note 3: Advantages of AsFlFFF include that the upper channel wall can be made of a transparent material, allowing the accumulation and sample migration to be viewed. Sample focusing can be achieved during relaxation, resulting in a narrower sample band being produced, resulting in lower band broadening.

Source: Adapted from [15]

4.2 Brownian field-flow fractionation (BFFF) mode

normal FFF mode (NFFF)
normal FFF

Mechanism of *field-flow fractionation* of sub-micron particles that relies on Brownian motion

Note 1: BFFF mode requires a specific sample injection procedure described as stop-flow injection or a relaxation step. A relaxation time is needed to equilibrate the sample under the influence of a force field and Brownian diffusion in the absence of flow. The injected sample is forced to concentrate in a thin layer adjacent to the accumulation wall (channel bottom).

Note 2: When the flow is switched on, *elution* begins with smaller particles, which are distributed further away from the accumulation wall and thus protrude further into the faster flow streams; larger particles elute later.

Note 3: Two main factors influencing the sample behaviour in BFFF mode are the properties of particles characterized by the diffusion coefficient and the strength of the cross-field applied.

Source: Adapted from [15]

4.3 coiled-tube field-flow fractionation (CTFFF)

Field-flow fractionation in a long (usually 10 m or more) rotating coiled column under the action of asymmetrical centrifugal field

Note 1: CTFFF can be attributed to non-conventional SdFFF, which enables the sample mass to be increased to gram levels.

Note 2: The complex asymmetrical force field is generated in planetary centrifuges where the column drum rotates around its own axis and at the same time revolves around the central axis of the centrifuge

Source: Adapted from [15]

4.4 field-flow fractionation (FFF)

Separation based on the retention and *elution* of macromolecular, colloidal, and particulate matter achieved by the combined action of the non-uniform flow speed (velocity) profile of a carrier fluid (mobile phase) and a physical force field (*e.g.* gravitational, centrifugal, electrical) applied at right angles to a thin (0.05 to 0.5 mm) channel. (see Fig. 5)

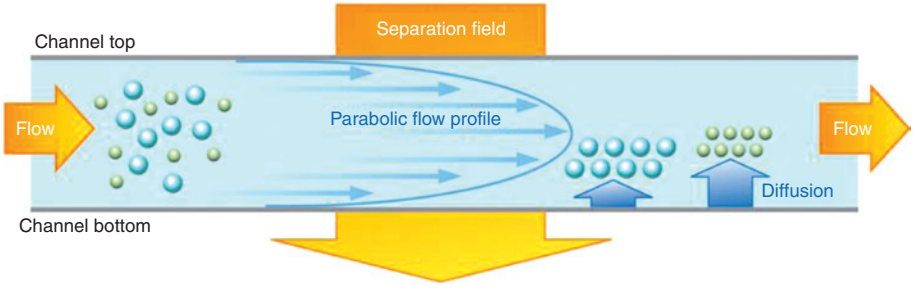

Fig. 5: Basic principle of *field-flow fractionation*.

Note 1: Although similar to *liquid chromatography*, FFF requires no *stationary phase*, so the separation channel contains no packing material and only physical interactions are involved in the separation process.
Note 2: FFF is applicable to the fractionation and characterization of colloidal and solid particles, polymers, and biological cells in the size range from 1 nm to 50 μm.
Note 3: The sample mass injected is usually less than 1 mg to avoid overloading.

Source: Adapted from [15]

4.5 flow field-flow fractionation (FlFFF, F4)

Field-flow fractionation in which the physical force field is a nonspecific hydrodynamic force field across the channel, generated by a secondary *mobile phase* cross-flow in addition to the normal channel flow.

Note 1: The sample components are driven by the cross-flow toward the accumulation wall, which consists of porous frit covered with a permeable membrane.
Note 2: Like SdFFF, FlFFF can be used for submicron particles and macromolecules using the *Brownian mode* or micron size particles utilizing the *hyperlayer mode*.
Note 3: This technique can be applied to the size fractionation of different synthetic polymers, liposomes, proteins, viruses, dissolved organic matter, humic and fulvic acids as well as their complexes with trace metals, diesel soot particles, marine colloids, heterogeneous and arbitrarily shaped natural colloidal particles, gold colloid standards starting from 5 nm.
Source: Adapted from [15]

4.6 gravitational field-flow fractionation (GFFF)

Field-flow fractionation in which the physical force field is the Earth's gravitational field.

Note 1: GFFF can fractionate particulate matter in the micrometre size range (large microorganisms, blood cells, sediments, etc).
Note 2: Since the Earth's gravitational field produces relatively weak forces, this mode is only appropriate for micron size particles, hence the separation is an example of *hyperlayer focusing field-flow fractionation*.
Source: Adapted from [15]

4.7 hyperlayer focusing field-flow fractionation (HyFFF) mode

hyperlayer field-flow fractionation mode
lift-hyperlayer field-flow fractionation mode

Mechanism of *field-flow fractionation* of particles greater than 1 μm in diameter relying on hydrodynamic lift forces near the channel wall to move particles into faster flowing streams.

Note 1: HyFFF is a preferred alternative to *steric field-flow fractionation*. The retention of particles larger than 1 μm typically increases with flow rate. Particles are driven away from the wall by a hydrodynamic lift force and elevated into faster flow streams. The particles form thin layers at an equilibrium position, where the external field is balanced by the opposing hydrodynamic lift force, as compared to steric mode, where the thin layer of particles is adjacent to the accumulation wall
Note 2: Due to the large size of the particles, Brownian diffusion away from the accumulation wall is assumed to be negligible in this mode.
Source: Adapted from [15]

4.8 sedimentation field-flow fractionation (SdFFF)

Field-flow fractionation in which the physical force field is a centrifugal force field.

Note 1: SdFFF is performed in a circular flat channel inserted inside a centrifugal basket.
Note 2: SdFFF can be applied to a wide range of colloidal and micron size particles and is a powerful method for cell separations, including industrial carbon blacks, silica nanoparticles, silver and gold nanoparticles, aquatic sediments, aerosols, and latex beads.
Note 3: SdFFF can be successfully applied to the fractionation and characterization (sizing) of submicron particles, starting from about 5 nm, but this limit depends on the particle density, as the separation is based on the buoyant mass of the particles.
Note 4: SdFFF can operate in both the Brownian (*BFFF*) and hyperlayer (*HyFFF*) modes and caution must be exercised if samples span the Brownian-hyperlayer transition point of about 1 μm, as this can lead to separated fractions containing two particle sizes, smaller particles separated by Brownian mode and larger particles separated by hyperlayer mode.
Source: Adapted from [15]

4.9 split-flow thin-cell fractionation (SPLITT)

Separation in which laminar flow conditions are established between a liquid containing suspended sample and a carrier liquid, with a physical force field applied across the streams.

Note 1: Although SPILTT is similar to *field-flow fractionation*, it should not be referred to as FFF. The separation is achieved by the combined action of controlled flow rates and an applied cross-field.
Note 2: The system has two channel inlets and two outlets. Sample suspension is continuously pumped into one inlet and carrier liquid is pumped into the other inlet. Due to the laminar flow conditions, these two streams do not mix.
Note 3: SPLITT enables the separation of samples of masses of the order of grams.
Note 4: Fields that have been tested so far include gravitational, centrifugal and magnetic.
Note 5: A semi-preparative binary separation can be generated with smaller particles eluting from one outlet and larger particles emerging from the other. Careful manipulation of the inlet and outlet flow rates and the field strength enables the cut-off diameter of the particles to be selected.
Source: Adapted from [15]

4.10 steric field-flow fractionation (StFFF) mode

Mechanism of *field-flow fractionation* of particles greater than 1 μm in diameter relying on proximity to the accumulation wall.

Note 1: In StFFF the *elution* order is opposite to that of *Brownian field-flow fractionation* and larger particles move ahead of smaller ones.
Note 2: It was originally considered that particles are in contact with the accumulation wall during the entire *migration time* and consequently the centre of larger particles are situated closer to the centre of the channel in the faster flow streams. This is never achieved in practice due to hydrodynamic lift forces away from the accumulation wall.
Source: Adapted from [15]

4.11 symmetrical flow field-flow fractionation

Flow field-flow fractionation in which the speed of *mobile phase* is constant along the length of the channel due to constant channel width.

Note 1: The width is constant except for triangular sections at each of the ends of the channel.
Note 2: Theory and data analysis are simplified by the assumption of constant flow.
Source: Adapted from [15]

4.12 thermal field-flow fractionation (TFFF)

Field-flow fractionation in which the physical force field is created by a temperature difference across the channel.

Note 1: One side of the channel is heated and the other side is cooled. A relatively small temperature difference between the top and bottom walls of the channel provides a high thermal gradient.
Note 2: TFFF has been mainly used for the separation of lyophilic synthetic polymers, including ultrahigh molecular weight polymers and microgels. However, some reports of other types of macromolecules and particles, including aqueous solvents, are present in the literature.
Source: Adapted from [15]

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6 Index of abbreviations

AsFIFFF	asymmetrical flow field-flow fractionation 4.1
AF4	asymmetrical flow field-flow fractionation 4.1
BFFF	Brownian field-flow fractionation 4.2
CAE	capillary affinity electrophoresis 3.1
CCC	counter-current chromatography 2.5.3
CE	capillary electrophoresis 3.3
CEC	capillary electrochromatography 3.2
CGE	capillary gel electrophoresis 3.4
CIEF	capillary isoelectric focusing 3.5
CITP	capillary isotachophoresis 3.6
CSE	capillary sieving electrophoresis 3.7
CTFFF	coiled-tube field-flow fractionation 4.3
CZE	capillary electrophoresis 3.3
ECC	electrokinetic capillary chromatography 3.10
EKC	electrokinetic chromatography 3.10
FFF	field-flow fractionation 4.4
FIFFF	flow field-flow fractionation 4.5
F4	flow field-flow fractionation 4.5
GC	gas chromatography 2.4.1
GFFF	gravitational field-flow fractionation 4.6
HILIC	hydrophilic-interaction liquid chromatography 2.5.7
HIC	hydrophobic-interaction chromatography 2.5.8
HPLC	high-performance liquid chromatography 2.4.2
HSCCC	high-speed counter-current chromatography (type-J) 2.10.2.4
HyFFF	hyperlayer focusing field-flow fractionation 4.7

IC	ion chromatography 2.5.9
LC	liquid chromatography 2.4.2
NACE	non-aqueous capillary electrophoresis 3.30
MECC	micellar electrokinetic capillary chromatography 3.26
MEECC	microemulsion electrokinetic capillary chromatography 3.27
MEEKC	microemulsion electrokinetic chromatography 3.27
MEKC	micellar electrokinetic chromatography 3.26
PLOT	porous-layer open-tubular column 2.7.16
SdFFF	sedimentation field-flow fractionation 4.8
SCOT	support-coated open-tubular column 2.7.18
SFC	supercritical fluid chromatography 2.4.3
SN	separation number (in chromatography) 2.8.7
SPLITT	split-flow thin-cell fractionation 4.9
StFFF	steric field-flow fractionation 4.10
TFFF	thermal field-flow fractionation 4.12
TLC	thin-layer chromatography 2.3.2
UHPLC	ultra-high-performance liquid chromatography 2.4.2
UPLC	ultra-performance liquid chromatography 2.4.2
WCOT	wall-coated open-tubular column 2.7.19

7 Index of symbols

A_c	cross-sectional area of the column 2.7.6
A_s	asymmetry factor (in chromatography) 2.7.20
d_c	column diameter 2.7.2
d_f	liquid-phase film thickness 2.7.10
E	electric field strength 3.9
f_M	modulation frequency (in comprehensive chromatography) 2.10.5.3
F_c	flow rate (in chromatography) 2.7.30
h	reduced plate height 2.8.6
H	plate height 2.8.4
H_{eff}	effective plate height 2.8.1
k	retention factor (in column chromatography) 2.7.38
k_e	retention factor (in size-exclusion chromatography) 2.10.6.6
k_{MEKC}	mass distribution ratio (in micellar electrokinetic chromatography) 3.24
k_{MEEKC}	mass distribution ratio (in microemulsion electrokinetic chromatography) 3.25
K_C	distribution ratio (in counter-current chromatography) 2.10.2.2
K_0	distribution constant (in size-exclusion chromatography) 2.10.6.5
I	retention index (in chromatography) 2.7.39
K_c	distribution constant (in chromatography) 2.7.33
L	column length 2.7.4
L_{eff}	effective length of the capillary 3.8
m_S	mass of the stationary phase (in chromatography) 2.7.11
N	plate number (in chromatography) 2.8.5
N_{eff}	effective plate number 2.8.2
n_M	modulation number (in comprehensive chromatography) 2.10.5.4
p_c	critical pressure 2.10.1.2
pH_{zone}	zone pH (in counter-current chromatography) *2.10.2.14*
P_M	modulation period (in comprehensive chromatography) 2.10.5.5

r	relative retention (in chromatography) 2.7.36
R_f	retardation factor 2.7.37
R_s	peak resolution (in chromatography) 2.8.3
R_{sp}	specific resolution (in size-exclusion chromatography) 2.10.6.11
SN	separation number (2.8.7)
t_0	retention volume (time) of an unretained compound (in size-exclusion chromatography) 2.10.6.7
t_{eo}	electroosmotic hold-up time (in capillary electromigration) 3.14
t_{ext}	extra-column volume (time) (in chromatography) 2.7.7
T_c	critical temperature 2.10.1.3
t_M	hold-up volume (time) (in chromatography) 2.7.34
t_m	migration time (in electrophoresis) 3.29
t_{mc}	migration time of micelles (in micellar electrokinetic chromatography) 3.28
t_R	retention volume (time) (in size-exclusion chromatography) 2.10.6.8
t_R	total retention volume (time) (in chromatography) 2.7.41
t'_R	adjusted retention volume (time) (in size-exclusion chromatography) 2.10.6.4
t'_R	adjusted retention volume (time) (in chromatography) 2.7.32
t_t	total mobile phase volume (time) (in size-exclusion chromatography) 2.10.6.9
$\boldsymbol{u}$, (u)	mobile-phase velocity (speed) (in chromatography) 2.7.31
$\boldsymbol{u}$	electroosmotic mobility (in capillary electromigration) 3.15
V_0	interparticle volume of the column (in chromatography) 2.7.9
V_0	retention volume (time) of an unretained compound (in size-exclusion chromatography) 2.10.6.7
V_0	interparticle volume of the column (in size-exclusion chromatography) 2.10.6.1
V_c	column volume 2.7.5
V_{ext}	extra-column volume (time) (in chromatography) 2.7.7
V_i	intraparticle volume of the column (in size-exclusion chromatography) 2.10.6.2
V_M	hold-up volume (time) (in chromatography) 2.7.34
V_M	mobile-phase volume (in counter-current chromatography) 2.10.2.6
V_R	total retention volume (time) (in chromatography) 2.7.41
V_R	retention volume (time) (in size-exclusion chromatography) 2.10.6.8
V'_R	adjusted retention volume (time) (in chromatography) 2.7.32
V'_R	adjusted retention volume (time) (in size-exclusion chromatography) 2.10.6.4
V_S	stationary-phase volume 2.7.17
V_S	retention volume of the stationary phase (in counter-current chromatography) 2.10.2.11
V_t	total mobile-phase volume in column (in size-exclusion chromatography) 2.10.6.3
V_t	total mobile phase volume (time) (in size-exclusion chromatography) 2.10.6.9
w_b	peak width base (in chromatography) 2.7.26
w_h	peak width at half height (in chromatography) 2.7.27

Greek symbols

a	separation factor (in chromatography) 2.7.40
β	phase ratio (in chromatography) 2.7.15
β_V	phase volume ratio (in counter-current chromatography) 2.10.2.8
ε	interparticle porosity (in chromatography) 2.7.8
ζ	electrokinetic potential 3.12
$\boldsymbol{\mu}$	electrophoretic mobility 3.18
$\boldsymbol{\mu_{eo}}$	electroosmotic mobility (in capillary electromigration) 3.15
$\boldsymbol{v}_{ep}$	electrophoretic velocity 3.19
$\boldsymbol{v}_{eo}$	electroosmotic velocity 3.16
$\boldsymbol{v}_{tot}$ (v_{tot})	total velocity (speed) of the analyte (in capillary electrophoresis) 3.33

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