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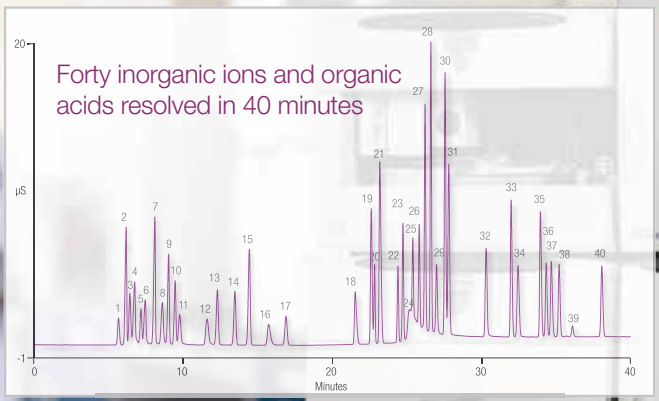

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The Chromatography and Sample Preparation Terminology Guide

Ronald E. Majors and John Hinshaw

In 2002, *LCGC* provided readers with a gas chromatography (GC) glossary to organize the myriad terms used in gas chromatography (1). Likewise, in 2008, the third glossary of common and not-so-common terms and "buzz words" for reference to high performance liquid chromatography (HPLC) columns and column technology was published (2). It is time for an update because new terms have arisen or, in some cases, their original meanings have expanded or changed. Because there are a number of terms common to both GC and LC, we decided to combine the glossaries into one large listing. In addition, with new sample preparation technologies also making their appearance, expanding the glossary to include terminology specific to sample preparation was another goal. Finally, ion chromatography (IC), which some feel is a subset of LC, does have some of its own nomenclature and so those terms are included here.

We stick to the conventions of the International Union of Pure and Applied Chemistry (IUPAC) in their "Nomenclature for Chromatography" that provides guidance and changes in some of the more commonly accepted terms (3). Still, there are many terms in common usage that are not in alignment with the IUPAC definitions and that nomenclature will be covered here as well.

This terminology guide is not intended to be an in-depth listing or highly theoretical coverage. For example, we have elected not to cover many of the myriad terms used in instrumentation, detection, data handling, and validation associated with chromatographic analysis but have chosen to use terms that may be encountered in everyday laboratory work around columns, injection techniques, phases, method development, sample preparation tasks, and general usage. The listing should be helpful to those just starting in chromatography but it can also serve as a refresher for long-time users in the field.

References

(1) J.V. Hinshaw, *LCGC* **20**(11), 1034–1040 (2002).
(2) R.E. Majors and P.W. Carr, *LCGC* **26**(2),118–168 (2008).
(3) L.S. Ettre, "Nomenclature for Chromatography" in *Pure and Appl. Chem.* **65**(4), 819–872 (1993).

96-well collection plate: A fixed-size polyethylene rectangular plate (127.8 mm × 85.5 mm): consisting of an array of 8 × 12 (96) small "test tubes" called *wells*; volumes of wells range from 0.5 to 2 mL.

96-well filtration plate: A fixed-size polyethylene rectangular plate (127.8 mm × 85.5 mm) consisting of an array of 8 × 12 (96) of small filter tubes (volumes range from 0.5 to 2 mL); a membrane filter placed at the bottom of the well is used to filter liquid samples; sometimes a prefilter is placed above the membrane filter to prevent clogging with particulate samples.

96-well plate: A small rectangular plastic plate consisting of 96 individual wells that are basically small-volume test tubes arranged in an 8 × 12 well pattern; used for liquid handling and other such requirements.

96-well solid-phase extraction plate: A small rectangular plastic plate consisting of 96 individual flow-through SPE wells arranged in an 8 × 12 array that have top and bottom frits to contain solid particles of sorbent or resin to perform SPE on a miniaturized scale; generally 1 mg to 0.2 g of packing is placed into the well, which can have a volume of up to 2 mL; used for automated SPE with *xyz* liquid handling systems or customized workstations.

A

A solvent: Usually the weaker solvent in a binary eluent or gradient elution separation. In reversed-phase liquid chromatography (LC), the A solvent typically is water or a water-rich mixture.

A* term:** The first term in the van Deemter equation. See ***eddy dispersion term and ***van Deemter equation.***

Absorption: The process of retention in which the solute partitions into a liquid-like coating.

Accelerated solvent extraction (ASE): Trade name for a pressurized fluid extraction system introduced by Dionex and now sold by Thermo Fisher Scientific; see ***pressurized fluid extraction*** for details of technique.

Active flow technology: A concept that incorporates two types of column designs: *curtain flow technology* means segmenting the flow at the injection end of the column to ensure the analyte sees the middle of the packed bed where it is not disturbed by wall effects; *parallel segmented flow* at the column outlet selects just the middle portion of the flow profile resulting in improved efficiency without the presence of wall effects, giving the best overall column efficiency; a special endfitting design is used to sample the center of the parabolic flow profile.

Active sampling: In active gas sampling, a pump is used to push the sample through a mass flow controller and into the canister. Additional sample can be collected, relative to the amount that can be collected by passive sampling, by pressurizing the canister with sample. Commonly the sample is pressurized to 103 kPa (15 psig), effectively doubling the sample volume.

Active site: A reactive or strongly attracting site on the surface of a chromatographic packing that may bind analytes or cause peak tailing; sometimes mobile phase additives (such as a competing base) can negate the effects of active sites.

Activity: In adsorption chromatography, the relative strength of the surface of the packing. For silica gel, the more available the silanol groups, the more active the surface. Activity can be controlled

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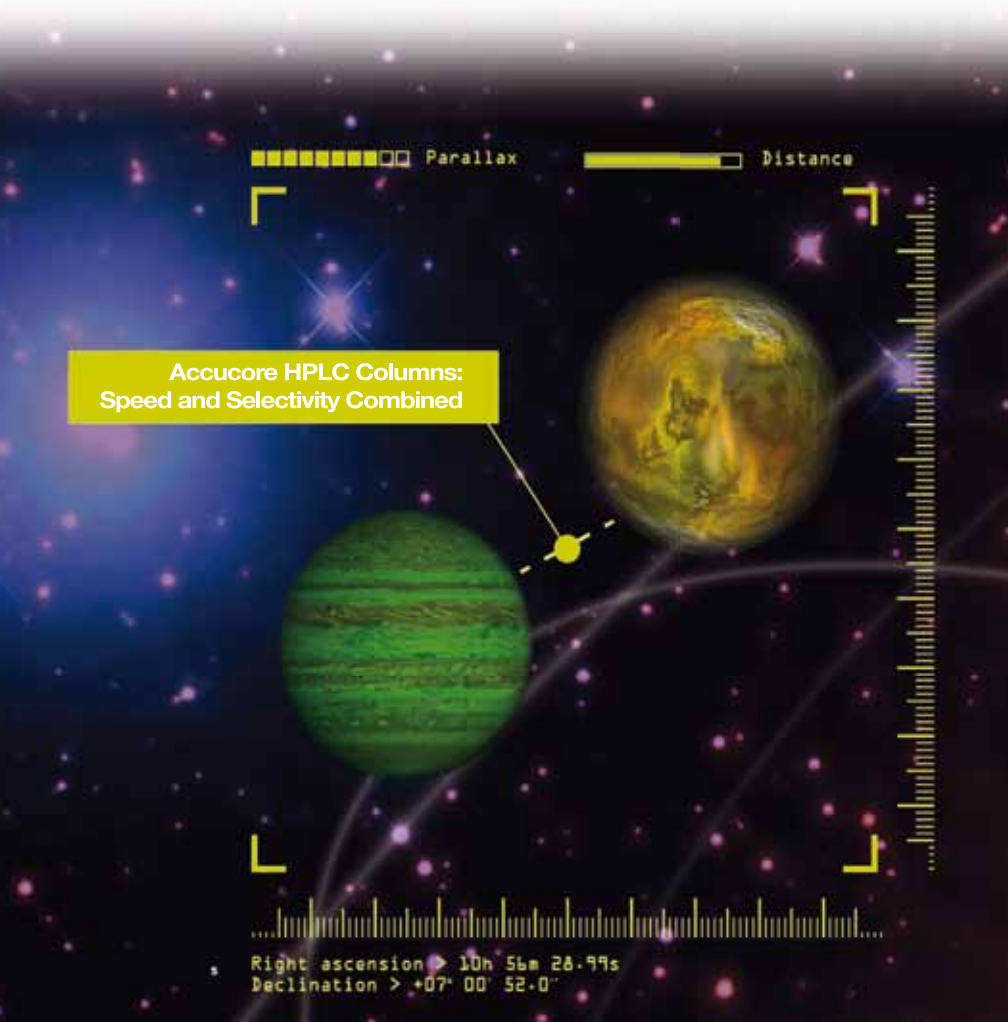

by the addition of water or other polar modifier that hydrogen-bonds to the active sites thereby reducing the surface activity; can also refer to biological activity of a biomolecule.

Additive: A substance added to the mobile phase to improve the separation or detection characteristics; examples would be a competing base to negate the effects of silanols, a chelating agent to block metal sites, or addition of a UV-absorbing compound to perform indirect photometric detection.

Adsorbent: Packing used in adsorption chromatography. Silica gel and alumina are the most frequently used adsorbents in chromatography and sample preparation.

Adsorption: A process of retention in which the interactions between the solute and the surface of an adsorbent dominate. The forces can be strong forces (for example, hydrogen bonds) or weak (van der Waals forces). For silica gel, the silanol group is the driving force for adsorption and any solute functional group which can interact with this group can be retained on silica. The term *adsorption* places emphasis on the surface versus penetration or embedding in the stationary phase coated or bonded to a surface.

Adsorption chromatography: One of the basic separation and SPE modes that relies on the adsorption process to effect a separation. Silica gel and alumina are the most frequently used normal-phase adsorbents in LC. Molecules are retained by the interaction of their polar function groups with the surface functional groups (for example, silanols of silica). Carbon is also used as an adsorbent in a reversed-phase LC mode. Porous polymer, carbonaceous, and molecular sieve packings in GC exhibit adsorptive properties as well.

Adsorption isotherm: In adsorption, a plot of the equilibrium concentration of sample in the mobile phase per unit volume versus the concentration in the stationary phase per unit weight. The shape of the adsorption isotherm can determine the chromatographic behavior of the solute such as tailing, fronting, or overload.

Aerogel: A packing prepared when the dispersing agent is removed from a gel system without collapsing the gel structure. Silica gels and glass beads used for SEC are examples of aerogels that can retain their structures even at the high pressure used in HPLC. See ***xerogels.***

Affinity chromatography: A technique in which a biospecific adsorbent is prepared by coupling a specific ligand (such as an enzyme, antigen, or hormone) for the macromolecule of interest to a solid support (or carrier). This immobilized ligand will interact only with molecules that can selectively bind to it. Molecules that will not bind are eluted unretained. The retained compound can later be released in a purified state. Affinity chromatography is normally practiced as an "on–off" separation technique.

Agarose: High-molecular-weight polysaccharide used as a separation medium in biochromatography. It is used in bead form and often used in gel filtration chromatography using aqueous mobile phases.

Alkoxysilane: A reactant used for the preparation of chemically bonded phases. It will react with silica gel as follows: $R_3SiOR + \equiv SiOH \rightarrow \equiv Si–OSiR_3 + ROH$ where R is an alkyl group.

Alumina: A normal-phase adsorbent used in adsorption chromatography. Aluminium oxide (Al_2O_3) is a porous adsorbent which is available with a slightly basic surface; neutral and acidic modifications can also be made. Basic

alumina can have advantages over silica, which is considered to have an acidic surface; alumina is seldom used as an HPLC column packing in practice. In GC applications, alumina will separate low molecular-weight gases.

Amino phase: A propylamino phase used in normal-phase chromatography. It is a somewhat reactive phase for any solute molecule (for example, aldehydes) or mobile phase additive that can react with amines. The amino phase has found some applications as a weak anion exchanger and for the separation of carbohydrates using a water–acetonitrile mobile phase. It is a relatively unstable phase.

Amperes full-scale (AFS): Extent of the maximum detector output, for detectors utilizing an electrometer.

Amperometric detection: Electrochemical detection applying a constant potential to the working electrode. Measured current from oxidation or reduction is proportional to the sample concentration. Very selective and sensitive method. Works with electrode reactions not changing the electrode surface (for example, cyanide, nitrite, thiosulfate, phenols). Only approximately 10% of the analyte is oxidized or reduced. May be used standalone as well as in series or parallel to other detectors.

Ampholyte: A substance that carries both positive and negative charges (they are amphoteric). Examples: amino acids, proteins.

Amphoteric ion-exchange resin: Ion-exchange resins that have both positive and negative ionic groups. These resins are most useful for ion retardation where all ionic materials can be removed from solution since the anionic and cationic functionalities coexist on the same material.

Analyte protectorant: In GC, a chemical compound or compounds that are added to a sample before injection to cut down on interactions between analytes that are unstable or behave poorly in the GC flow path on active sites; the protectorants are chosen so that they do not interfere with the analysis of the compounds of interest yet prevent these compounds from interacting with the active sites in the flow path; these protectorants are not generally required for LC and LC–MS.

Analytical column: A chromatography column used for qualitative and quantitative analysis; a typical analytical column for LC will be 50–250 cm × 4.6 mm, but columns with smaller diameters (down to 0.05 mm i.d.) can also be considered as analytical columns. GC analytical columns range in length from 1 m to as much as 60 m, with inner diameters ranging from less than 100 μm up to 2 mm. Stationary phases can be coated or bonded onto the interior of the tubing; packed GC columns are generally wider and shorter and are less frequently used nowadays. Chromatography columns can be constructed of stainless steel, glass, glass-lined stainless steel, PEEK, fused silica, and other metallic and nonmetallic materials.

Anion exchange: The ion-exchange procedure used for the separation of anions. Synthetic resins, bonded phase silicas, and other metal oxides are available for this mode. A typical anion-exchange functional group is tetraalkylammonium, making a strong anion exchanger. An amino group on a bonded stationary phase would be an example of a weak anion exchanger.

Argentation SPE: The incorporation of a silver salt into the SPE stationary phase will help in retaining compounds with olefinic bonds. Normally used in

organic solvents to maximize charge-transfer interactions.

Array 96-well plate: A 96-well SPE plate where the 96 individual wells are removable from the base plate; such a setup allows users to place different types and amounts of SPE sorbents into various configurations in each of the 96-wells. This type of 96-well plate has also been referred to as a *flexible 96-well plate configuration.*

Asymmetry: Factor describing the shape of a chromatographic peak. Theory assumes a Gaussian shape and that peaks are symmetrical. A quantitative measure is the peak asymmetry factor, which is the ratio of the distance from the peak apex to the back side of the chromatography curve over the distance from the peak apex to the front side of the chromatography curve at 10% of the peak height. Various other measures of asymmetry are in common use, especially the USP method. See also ***Foley–Dorsey equation.***

Asymmetry factor: A factor that denotes band shape; calculated from the chromatographic peak by dropping a perpendicular at the peak apex and a horizontal line at 10% of the peak height; at the intersection the distance to the tail of the peak along the horizontal line (distance B) divided by the distance along the horizontal line to the front of the peak (distance A); this ratio is the peak asymmetry factor; for a symmetrical peak the value is one; for a fronting peak the value is less than one; for a tailing peak, the value is greater than one; the higher the value the less symmetrical the peak is; values greater than 2 are generally unacceptable.

Atmosphere (atm): A unit of pressure. 1 atm = 101.325 kPa or 14.6959 psi.

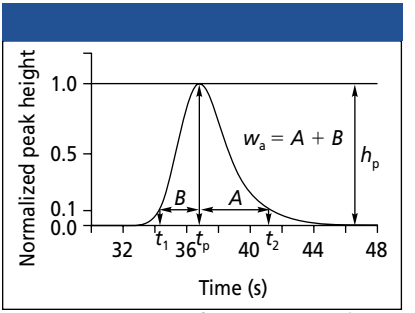

Figure 1: Example of a tailing peak. (Modified with permission from reference 3.)

Average particle size (d_p): The average particle size of the packing in the column. A 5-µm LC column would be packed with particles with a definite particle size distribution because packings are never monodisperse. Particle sizes in GC usually are expressed in terms of mesh size distribution; 80–100 mesh is a commonly used particle range. See ***particle size distribution.***

B

B solvent: Usually the stronger solvent in a binary eluent or gradient separation. In reversed-phase LC, typically the organic modifier or modifier-rich binary mixture with water.

B* term:** The second term of the van Deemter equation; the first term of the Golay equation. See ***longitudinal diffusion, molecular diffusion term, van Deemter equation, Golay equation.

Back extraction: Used in liquid–liquid extraction to perform an additional extraction to further purify a sample; initially the extraction may take place with an aqueous solvent buffered at a high pH and an immiscible organic solvent; after the initial extraction takes place and interferences are removed, then by having another aqueous solution at a low pH, one can back-extract the analyte into the organic layer based on the analyte now

being in a neutral form. An example would be for the cleanup of an acidic substance containing –COOH groups; at high pH the carboxyl would be ionized and prefer the aqueous layer and impurities may migrate to the organic phase and discarded; then the pH of the aqueous layer can be adjusted to a low value. Now the carboxyl group is in an unionized form and readily extracted into the organic layer as a purified substance.

Backflushing: Useful in chromatography to remove compounds that are held strongly at the head of a column. By reversing the flow at the conclusion of a run, analytes trapped at the head of the column can be flushed from the column entrance because they have a shorter distance to travel; sometimes a strong solvent in LC or elevated temperatures in GC will be needed to move them along. A valve or fluidic device is used to effect the change of mobile-phase flow direction. Backflushing can be used for analysis of these compounds or merely to remove them from the column.

Back-pressure regulator: In LC, a device placed on-line after the detector to maintain a positive pressure on the flow cell minimizing solvent outgassing problems in the detector. In GC, the term usually refers to a carrier-gas regulator in the split vent line that maintains a constant pressure at the inlet as split flows change.

Bakeout: The process of removing contaminants from a column by operation at elevated temperatures, which should not exceed the maximum column temperature.

Band: Refers to the chromatographic peak as it moves along and is eluted from the column.

Band broadening: The process of increase in width and concomitant dilution of the chromatographic band as it moves down the column. The peak is injected as a narrow slug and ideally each separated component would elute as a narrow slug of pure compound if not for the process of band broadening. The measure of band broadening is the peak dispersion, σ, or more correctly *N*, the number of theoretical plates in the column. Sometimes called *band dispersion* or *band spreading*.

Band width: See ***peak width at base*** and ***peak width at half-height.***

Baseline: The baseline is the line drawn by the recording device representing the signal from the detector when only mobile phase is passing through, in the absence of any solutes. It also represents the point from which calculations are often made on peaks to determine peak area or peak height.

Baseline drift: Term for any regular change occurring in baseline signal from an LC or GC detector; it may arise from changes in flow rate of the mobile phase or from stationary phase bleed and may trend in a positive or negative direction. Baseline drift occurs over a longer period of time than baseline noise.

Baseline noise: Irregular variations (short term) in the chromatographic baseline as a result of electrical noise or temperature fluctuations, outgassing in the flow cell, or poorly mixed mobile-phase solvents.

Bed volume: See ***column volume.***

BEH: Bridged ethyl hybrid; an inorganic–organic HPLC particle; has higher pH limits than silica gel.

BET method: A method for measuring surface area developed by Bruner, Emmett, and Teller (BET) that uses nitrogen adsorption–condensation in pores at liquid nitrogen temperature. Pore volume

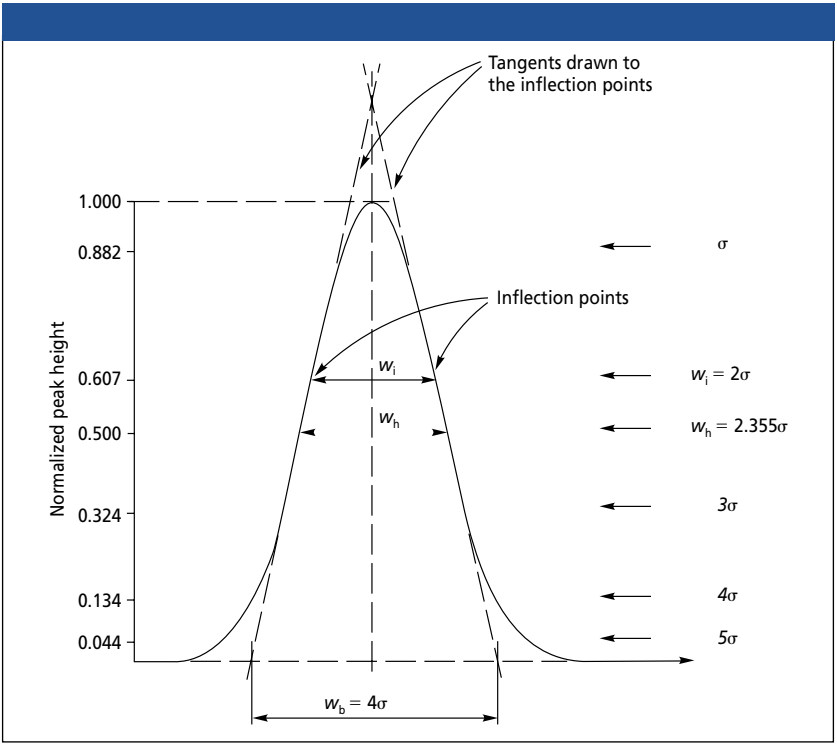

Figure 2: Widths of a Gaussian peak at various heights as a function of the standard deviation σs) of the peak. (Modified with permission from reference 2.)

and pore size distribution can also be obtained from BET calculations.

Bidentate silane: A specific type of bonded phase in which a short hydrocarbon bridge connects two silicon atoms in a silane that is bounded to the surface through two siloxane groups.

Bimodal: In SEC, can be a porous packing material that has two distinct pore sizes or pore size distributions; in ion-exchange or HILIC chromatography or sample preparation can be a packing material that has two types of functionalities (for example, cation exchange and reversed phase; cation and anion) on one packing; in some cases, mixed beds consisting of two different packings in one column can be bimodal.

Binary mobile phase: Mobile phase consisting of two solvents or buffers (or one of each).

Bind–elute : In SPE, the normal mode of operation where upon loading the sample onto a conditioned sorbent or resin, the analytes of interest are retained (bound) while interferences and perhaps some of the matrix is not retained by the packing; after a wash step to remove some of the undesired sample components, the elution step uses a strong solvent to elute the analytes of interest in a small volume.

Biocompatible: A term to indicate that the column or instrument component will not irreversibly or strongly adsorb or deactivate biomolecules, such as proteins. Frequently means metal-free or ceramic surfaces and components.

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Blank: More correctly named *method blank*. A blank prepared to represent the matrix as closely as possible. The method blank is prepared and analyzed exactly like the field samples. Purpose: Assess contamination introduced during sample preparation activities.

Bleed: Loss of material from a column or septum due to high-temperature operation. May result in ghost peaks plus increased detector baseline offset and noise; in extreme cases, bleeding chemicals from the stationary phase may build up on detector surfaces.

Blending: Refers to the process of making a heterogeneous sample into a more consistent and uniform sample by some type of blending operation; the most popular type of blender is the mechanical blender that chops a semisoft material into smaller parts.

Bonded phase: A stationary phase that has been chemically bonded to the inner wall of an open-tubular (capillary) column or to the support particles. In LC, the substrate is usually a silica gel particle or other base material.

Bonded-phase chromatography: The most popular mode in LC, in which a phase chemically bonded to a support is used for the separation. The most popular support for bonded-phase chromatography is microparticulate silica gel and the most popular type of bonded phase is the organosilane, such as octadecyl (for reversed-phase chromatography). Approximately 70% of all HPLC is carried out on chemically bonded phases.

Bonded-phase concentration: See ***coverage.***

Boxcar chromatography: See ***column switching***; alternate name.

Breakthrough capacity: See ***breakthrough volume.***

Breakthrough volume: The volume at which a particular solute pumped continuously through a column will begin to be eluted. It is related to the column volume plus the retention factor of the solute. It is useful to determine the total sample capacity of the column for a particular solute.

BTEX: Refers to benzene, toluene, ethylbenzene, and xylenes analysis.

Buffer: A solution that maintains constant pH by resisting changes in pH as a result of dilution or addition of small amounts of acids and bases.

Buffer capacity: A quantitative measure of the potential of a buffer solution (defined as the number of equivalents of strong acid or base to cause a one unit change in the pH of 1 L of a buffer solution) or simply the ability of a buffer to withstand injections of a buffered sample solution without a change in mobile-phase pH; capacity determined by pH, buffer pK_a, and buffer concentration.

Buffer strength: See ***ionic strength.***

C

C4, C8, C18: Refers to the alkyl chain length of a reversed bonded phase.

***C* term:** The interphase mass transfer term of the van Deemter and Golay equations.

Canister collection: A stainless steel vessel designed to hold vacuum to less than 1.3 Pa (10 mTorr) or pressure to 275 kPa (40 psig). Canisters are available in a range of volumes: 400 mL, 1.0 L, 3.0 L, 6.0 L, and 15 L. The size of canister used usually depends on the concentration of the analytes in the sample, the sampling time, the flow rate, and the sample volume required for the sampling period. Typically, smaller canisters are

used for more concentrated samples, such as soil gas collection, 3-L and 6-L canisters are used to obtain integrated (TWA) ambient air samples at sampling times of up to 24 h, and large 15-L canisters are used for reference standards. Sampling time will be limited by the combination of canister size and the flow rate at which the sample is to be collected.

Capacity: See ***sample capacity.***

Capacity factor (*k'*): Deprecated name for ***retention factor.***

Capillary column: Refers to chromatography columns of small inner diameter, ostensibly small enough to display a capillary effect with liquids. The diameter below which a column is considered "capillary" is poorly defined. See ***open-tubular column, capillary LC***.

Capillary column, packed: A capillary column that is packed with stationary-phase particles. In GC, 1/16-in. o.d. by 1-mm i.d. columns are common.

Capillary electrochromatography (CEC): A hybrid technique where capillary columns are packed with chromatographic sorbents and electroosmotic flow moves mobile phase through the column rather than pressure; the technique has the surface-mediated selectivity potential of HPLC and the high efficiency of CE.

Capillary GC: See ***open-tubular column***.

Capillary LC: Generally refers to HPLC carried out in a fused-silica or other type of capillary column; most of the time the dimensions are in the sub-0.5-mm i.d. range. Has also been called ***micro LC***.

Capillary micellar electrochromatography (CMEC): The CEC version of MEKC.

Capillary tubing: Tubing to connect various parts of the chromatograph in order to direct flow to the proper place. Most capillary tubing used in HPLC is less than 0.020 in. in internal diameter. The smallest useful internal diameter is about 0.004 in.

Capping: Same as ***endcapping***.

Carbon load: For a bonded-phase silica, term usually used to describe the surface coverage or the degree to which the available silanols on the column packing's surface have reacted and been replaced with the bonded phase; the higher the carbon load, the lower number of residual silanols. The carbon load is normally expressed as % carbon (for example, 12% carbon). In reversed-phase LC, the higher the carbon load, the greater the analyte retention.

Carrier: A term most often used in affinity chromatography; refers to the support that is used to attach the active ligand, usually by a covalent bond. Can also refer to the support in other chromatography modes, such as LLC.

Carrier gas: Term for the gaseous mobile phase in GC.

Cartridge: Generally refers to the container used in SPE or filtration; a cartridge may be as simple as a medical-grade syringe barrel that is filled with packing contained at both ends by frits; it can also be a molded device or even a stainless steel device that contains similar sorts of packing material. In SPE, the device is also referred to as an SPE tube.

Cartridge column: A column type that has no endfittings and is held in a cartridge holder. The column consists of a tube and the packing is contained by frits in each end of the tube. Cartridges are easy to change and are less

expensive and more convenient than conventional columns with endfittings.

Cation-exchange chromatography: The form of ion-exchange chromatography that uses resins or packings with functional groups that can separate cations. An example of a strong cation functional group would be a sulfonic acid; a weak cation-exchange functional group would be a carboxylic acid.

Centrifugation: Centrifugation is a process that involves the use of the centrifugal force for the sedimentation of mixtures with a centrifuge (see ***centrifuge***). This process is used to separate two immiscible liquids. More-dense components of the mixture migrate away from the axis of the centrifuge, while less-dense components of the mixture migrate toward the axis. Chemists and biologists may increase the effective gravitational force on a test tube so as to more rapidly and completely cause the precipitate ("pellet") to gather on the bottom of the tube. The remaining solution is properly called the "supernate" or "supernatant liquid." The supernatant liquid is then either quickly decanted from the tube without disturbing the precipitate, or withdrawn with a Pasteur pipette.

Centrifuge: A centrifuge is a piece of equipment, generally driven by an electric motor (some older models were spun by hand), that puts an object in rotation around a fixed axis, applying a force perpendicular to the axis (see ***centrifugation***).

Certify: Specific SPE products for drugs of abuse isolation and analysis.

Chain length: The length of carbon chain in the hydrocarbon portion of a reversed-phase packing. It is expressed as the number of carbon atoms (for example, C8, C18). Specifically excludes the short chains typical methyl, isopropyl, and *sec*-butyl groups also attached to the silane.

Channeling: Occurs when voids created in the packing material cause mobile phase and accompanying solutes to move more rapidly than the average flow velocity allowing band broadening to occur. The voids are created by poor packing or erosion of the packed bed.

Charged aerosol detection (CAD): The effluent from the LC column is nebulized and then vaporized in a heated drift tube, which results in a cloud of analyte particles; these particles are charged and then the current from the charged particle flux is measured. The ELSD technique measures the light scattering properties of the aerosol particles. CAD is more sensitive and gives a more linear response than ELSD; it is also a universal detection method.

Check valve: A device inserted into a moving fluid stream that allows flow of the stream in only one direction; most often used on the inlet and outlet sides of an HPLC pump.

Chelating resin: Chelating resin contains functional groups that will interact with cationic species (for example, metals such as copper, iron, heavy metal ions); useful for concentrating trace quantities or for separation.

Chemical filtration: A liquid sample is passed through a packing material (for example, adsorbent, ion exchange, and so forth) that selectively interacts with one or more compounds within the sample and acts as a chemical way to remove and purify the liquid sample. Regular filtration does not involve any chemical interaction but merely removes particulates.

Chemical suppression: Remove background conductivity by ion exchange. Converts the eluent into a low- or non-conducting component (for example, carbonate into carbonic acid, hydroxide into water, or nitric acid into water). Anion analysis: the counter cation (for example, sodium) is replaced by the proton (H^+). Cation analysis: the counter anion (for example, nitrate) is replaced by hydroxide (OH^-). The measured signal is the corresponding acid or base of the anion or cation respectively. The dissociation of these acids and bases influences the signal. All suppressor devices work on this principle. The typical background conductivity after suppression is <20 μS/cm.

Chemisorption: Sorption due to a chemical reaction with the packing. Most such interactions are irreversible. Usually occurs on packings with reactive functional groups such as silanol or bonded amino phases. Chemisorption is common with metal oxide phases that have strong Lewis acid sites.

Chip format: A miniaturization technique where small channels on a glass, polymer or other type of matrix are used instead of large bore columns, capillaries, and so forth. The resulting format is greatly reduced in size compared to conventional chromatographic instruments; advantages are the reduction in sample, solvent, and so forth; can be more easily coupled to detection techniques (for example, MS and MS-MS) where smaller amounts of mobile phase can lead to sensitivity enhancements and reduction in ion suppression.

Chiral recognition: The ability of a chiral stationary phase to interact differently with two enantiomers leading to their chromatographic separation.

Chiral stationary phase (CSP): A stationary phase that is designed to separate enantiomeric compounds. The phase can be coated or bonded to solid supports, created in situ on the surface of the solid support, or can include surface cavities that allow specific interactions with one enantiomeric form.

Chlorosilane: A chemical reagent used to prepare siloxane bonded phases; reactivity changes from a monochlorosilane < dichlorosilane < trichlorosilane; the alkyl portion (for example, octadecyl, octyl, and so forth) will dictate the hydrophobicity of the resultant bonded phase; alkoxysilanes can be used but are less reactive.

Chopping: The process of mechanically cutting a sample into smaller parts.

Chromatogram: A plot of detector signal output versus time or elution volume during the chromatographic process.

Chromatograph: (*n*) A device used to implement a chromatographic separation; (*v*) the act of separation by chromatography.

Chromatographic conditions: Those chromatographic method experimental parameters that describe how an analysis was performed. Sufficient information must be presented so that the analysis can be duplicated for verification purposes.

Classification: The process of sizing column packing particles; generally, in HPLC a small particle size distribution provides better efficiency and a greater permeability because of the absence of fines. Classification can be performed by sedimentation, elutriation, and using centrifugal air classifiers.

Co-ion: An ion of the same sign of charge as the ionic groups making up the stationary phase.

Coating efficiency (CE, UTE, UTE%): A metric for evaluating column quality. The minimum theoretical plate height divided by the observed plate height: $CE = H_{min}/H$

Cold injection: An injection that takes place at temperatures below the final oven temperature, usually at or below the solvent boiling point.

Column: The tube and stationary phase through which mobile phase flows resulting in a chromatographic separation.

Column chromatography: Any form of chromatography that uses a column, tube, or plate to hold the stationary phase. Open-column chromatography, HPLC, and open-tubular capillary gas chromatography are all forms of column chromatography. Most often refers to open-column chromatography used for preparative work.

Column dead time: See ***hold-up time***.

Column equilibration: To provide reproducible results, a column should be equilibrated with the surrounding environment be it a temperature condition, mobile phase equilibrium, pressure condition, and so forth; in GC, it is important that the temperature of the column be stabilized after a temperature programmed run and in LC, the column must be returned to its original conditions before another gradient is run.

Column inner diameter (d_c): The inner diameter of an uncoated chromatography column.

Column length (L): The length of the analytical chromatography column used to perform the chromatographic separation. Distinct from the length of a precolumn (LC) or retention gap (GC) connected in series.

Column outlet flow rate, corrected (F_a): In GC, the column outlet flow rate corrected from column temperature and outlet pressure to room temperature and pressure, for example, the flow rate as measured by a flow meter. Difficult to measure directly for narrow-bore open-tubular columns, the flow rate can be calculated from the average carrier-gas linear velocity, pressure drop, temperatures, and the column dimensions: $F_a = (\bar{u}\pi d_c^2 T_0)/(4jT_c)$. Such calculations are the basis of electronic pressure control.

Column overload: If one exceeds the sample capacity (or loading capacity) of a column, peaks will become distorted and may be difficult to measure and to achieve reproducible chromatography from run to run. One can measure column capacity by running a breakthrough study (see ***breakthrough volume***).

Column packing: The solid material, usually a porous solid with or without a chemically interactive surface, placed inside or on the walls of the column used to differentially retain analytes; also referred to as the ***stationary phase;*** common packings include unbonded and bonded silica, resins, inorganic–organic hybrids, graphitized carbon, porous polymers, and molecular sieves.

Column performance: Denotes the column efficiency. See ***theoretical plate***.

Column plate number: Denotes the column efficiency. See ***theoretical plate***.

Column suppressor: Initial setup. Packed ion-exchanger columns were used for chemical suppression. Drawbacks: require regeneration, changes

of the selectivity throughout the usage.

Column switching: The use of multiple columns connected by switching valves to effect better chromatographic separations or for sample cleanup. Fractions from a primary column can be switched to two or more secondary columns which in turn can be further diverted to additional columns or to the detector (or detectors); sometimes referred to as ***multidimensional chromatography.***

Column temperature (T_c): Temperature of the column. A uniform temperature across the column usually is desirable; however, GC separations also may be performed with a moving temperature gradient along the column length.

Column volume (V_c): The volume of the unpacked, uncoated column: $V_c = A_c L = \pi r_c^2 L$, where A_c and L are the cross-sectional area of the tube and the tube length, respectively.

Cool-down time: Length of time required to cool a GC oven from the final oven temperature to the initial oven temperature. Shorter cool-down times allow a greater number of analyses to be performed in a given time period.

Competing base: In reversed-phase LC, addition of a small basic compound such as triethylamine or dimethyloctylamine at 25–50 mM concentration to the mobile phase to inhibit basic analytes from interacting with residual silanols; works by law of mass action because the concentration of competing base is much greater than that of the analyte. See also ***additive.***

Comprehensive GC (GC×GC): Two-dimensional technique in which all compounds experience the selectivity of two columns connected in series by a retention modulation device, thereby generating much higher resolution than with any single column.

Comprehensive two-dimensional chromatography: Two-dimensional chromatography applied to every fraction. See ***two-dimensional chromatography***.

Compressibility correction factor (j): Due to gas compressibility, the carrier gas expands and its velocity increases as it proceeds along a GC column from the inlet pressure p_i to the outlet pressure p_o. The carrier gas compressibility correction factor corrects the carrier gas velocity at the outlet of a GC column to the average carrier gas velocity: $j = 3(P^2 - 1)/2(P^3 - 1)$, where P is the column pressure drop: $P = p_i/p_o$

Concentration: The process of increasing the strength or density of a diluted sample; a more concentrated sample will be easier to measure; concentration can be accomplished by a wide variety of sample preparation techniques such as evaporation, adsorption, diffusion, and so forth.

Conditioning (SPE): This is generally considered to be the first step in SPE; the stationary phase must first be put into a chemical or physical state that it can accept the sample solution loaded in the second SPE step; a conditioning solvent is passed through the SPE stationary phase where it will solvate the phase so that it will more easily sorb the sample of interest; for a reversed-phase SPE cartridge, methanol or acetonitrile serves as a conditioning solvent; sometimes the excess conditioning solvent must be removed but the packing shouldn't be allowed to dry out because that may affect the "conditioned" phase.

Conductivity: Conductivity is the inherent parameter of all ions. Therefore it is the signal for measuring the chromatogram. It may be measured directly or after chemical and sequential suppression respectively. As conductivity is strongly dependent on the temperature (around 2 %/°C), a thorough isolation and thermal stabilization of the detector block is recommended. The measured conductivity of a solution is given by the sum of the "single ion conductivities." The single ion conductivity is a linear function of the concentration of the ion and its equivalent conductivity. At very low concentrations or at constant ion strength the equivalent conductivity is constant. The measured signal in nonsuppressed IC is proportional to the concentration and the difference of eluent and sample ion: As the ionic strength of the solution is constant (stoichiometric ion exchange) also the equivalent conductivities are constant. This leads to very linear calibration curves. The measured signal in suppressed IC is proportional to the sum of the equivalent conductivities of the analyte ion and the counterion (which has been introduced by suppression). As only the sample ion and its counterion is adding to the measured conductivity after suppression, the total concentration is changing during the peak. Therefore the equivalent conductivities are no longer constant. This is one reason for the inherent nonlinearity of the calibration curves in suppressed IC.

Coning and quartering: A sample size reduction technique where a portion of free-flowing solid material (powder) is systematically divided into quadrants to achieve a statistically representative sample. Coning and quartering is a method used by analytical chemists to reduce the sample size of a powder without creating a systematic bias. The technique involves pouring the sample so that it takes on a conical shape, and then flattening it out into a cake. The cake is then divided into quarters; the two quarters that sit opposite one another are discarded, and the other two are combined and constitute the reduced sample. The same process is continued until an appropriate sample size remains. Analyses are made with respect to the sample left behind.

Continuous liquid–liquid extraction: Useful when the K_D value is very low or the required sample volume is very large when multiple extractions are impractical; also if the extraction is slow, a long time may be required for equilibrium to be established; in continuous LLE, fresh solvent is continually recycled through the aqueous sample; continuous extractors are available for heavier-than-water and lighter-than-water solvents.

Controlled surface porosity support: Same as ***porous layer bead*** and pellicular stationary phase.

Cool on-column injection: Cool on-column injection is a technique of introducing a sample as a liquid directly into a GC column; this lack of prior vaporization offers the following advantages: It eliminates sample discrimination; it eliminates sample alteration; and it provides high analytical precision. However, there are some special requirements: It requires relatively clean samples; real samples are often too concentrated for on-column injection and must be diluted; and peak splitting or peak distortion can occur due to differing polarities of solvent, stationary phase, and solutes.

Core–shell: See ***superficially porous particles (SPPs)***.

Coulometric detector: Same as amperometric detection, but with a conversion rate of 100%. All the analyte is oxidized or reduced at the working electrode. The response is larger than with amperometric detection. But on the other hand also the baseline noise is larger. Therefore the detection limits are almost the same.

Counterion: In an ion-exchange process, the ion in solution used to displace the ion of interest from the ionic site. In ion pairing, it is the ion of opposite charge added to the mobile phase to form a neutral ion pair in solution.

Coupled columns: A form of column switching that uses a primary column connected to two secondary columns via a selector valve. Fractions from column one can be selectively transferred to columns two and three for additional separation to occur. The term is also used to describe two or more columns connected in series to provide an increased number of plates.

Coverage: Refers to the amount of bonded phase on a silica support in bonded phase chromatography. Coverage is usually described in μmol/m^2 or in terms of %C (w/w).

Crash plate: Refers to the process of precipitating protein from plasma by the addition of a miscible organic solvent such as acetonitrile; when a 96-well flow-through or fixed-well plate is used for this process, it is referred to as *crashing* and the plate a *crash plate*.

Critical micelle concentration (CMC): The concentration of an ionic surfactant above which a micelle is formed by aggregation; micelles added to the mobile phase are used to improve the separation of nonionic substances in HPLC and CE (MEKC) by a partitioning mechanism.

Cross-linked phase: A stationary phase that includes cross-linked polymer chains. Usually it is also bonded to the column inner wall. See ***bonded phase***.

Crosslinking: For resins, during the process of copolymerization to form a three-dimensional matrix a difunctional monomer is added to form crosslinkages between adjacent polymer chains. The degree of cross-linking is determined by the amount of this monomer added to the reaction. For example, divinylbenzene is a typical cross-linking agent for the production of polystyrene ion-exchange resins. The swelling and diffusion characteristics of a resin are governed by its degree of cross-linking.

Crushing: Tungsten carbide variable jaw crushers for reducing the size of large, extremely hard, brittle samples.

Curtain flow technology: Curtain flow technology refers to the process of injection of sample across a radial cross section of an HPLC column to ensure the analyte sees the middle portion of the packed bed and not the wall where flow effects may be different; the technique is coupled with a parallel segmented flow fitting at the column outlet to select just the middle portion of the flow profile resulting in improved efficiency without the presence of wall effects.

Cutting: Cutting mills can reduce soft-to-medium hard materials (diameter < 100 mm).

Cyano phase: A chemically bonded phase that terminates with the -CN functional group; it can be used in normal-phase chromatography as a moderate polarity sorbent and in

reversed-phase chromatography as a short chain bonded phase.

Cyclodextrins: Cyclic oligomers of several D-(+)-glucopyranose units used in chiral HPLC and CE separations; popular ones are named α-, β-, and γ-CDs; they have a truncated cone shape, a relatively hydrophobic cavity, and primary and secondary hydroxyl groups at their end; separate on basis of differential inclusion of enantiomers; modified CDs with derivatized hydroxyl groups are also used for selectivity modification.

D

Data acquisition rate: A term referring to the rate of sampling of a detector output. To characterize a chromatographic peak at least 20–30 data points must be collected. The data acquisition rate, usually measured in hertz, defines how many data points per second are collected while the peak is moving through the detector. For fast chromatography, the data acquisition rate must be sufficiently rapid to characterize a narrow peak. Modern detectors have data rates up to 200 Hz; also known as *data rate* and *sampling rate*. See ***detector time constant***.

Dead volume: Dead volume is extra volume experienced by solutes as they pass through a chromatographic system, in particular any unswept volume exposed to the mobile phase flow. Excessive dead volume causes additional peak broadening. Related to the hold-up volume, which is the volume of mobile phase necessary to elute an unretained compound. See ***hold-up volume***.

Deep-well plate: A 96-well plate capable of handling up to 2 mL of liquid volume per well.

Degassing: The process of removing dissolved gas from the mobile phase prior or during use. Dissolved gas that may come out of solution in the detector cell can cause baseline spikes and noise. Dissolved air can affect certain detectors, such as electrochemical (by reaction) or fluorescence (by quenching). Dissolved gases can also cause pumps to lose prime. Degassing is carried out by heating the solvent or by vacuum (in a vacuum flask), or on-line using evacuation of a tube made from a gas-permeable substance such as PTFE, or by helium sparging.

Denaturing HPLC: Use of reversed-phase HPLC to investigate genetic mutations by the investigation of DNA base pairs.

Derivatization: A technique used in chemistry that transforms a chemical compound into a product (the reaction's derivate) of similar chemical structure, called a derivative. Generally, a specific functional group of the compound participates in the derivatization reaction and transforms the compound into one with a different reactivity, solubility, boiling point (volatility), melting point, aggregate state, or chemical composition. The resulting new chemical properties can be used for quantification (for example, UV or fluorescence detection) or better separation properties.

Desalting: Technique where low-molecular-weight salts and other compounds can be removed from nonionic and high-molecular-weight compounds. The use of a reversed-phase packing to retain sample compounds by hydrophobic effects yet allow salts to pass through unretained would be an example of desalting. The use of an SEC column to exclude large molecules

and retain lower-molecular-weight salts is another example. Desalting using dialysis is commonly used in protein purification.

Desorption: The process in chromatography where a molecule residing on the surface of a packing material or on another solid surface (for example, column wall or frit) or stationary phase moves from the surface into the mobile phase.

Detector response time: Time for a detector to respond to ~90% of the incoming solute amount. The response time is generally taken as 2–4 times the time constant. See also ***detector time constant.***

Detector time constant (τ): The time for a detector to respond to 1/e = 63.2% of an instantaneous change in solute amount. In general the detector time constant should be less than 10% of the peak width at half-height. Excessive detector dead volume, slowly responding electronics, digital data acquisition speeds, and signal filtering strongly influence detector response times. Too-slow detector response times cause peak tailing, loss of peak height and detectability, plus loss of peak resolution for closely adjacent peaks.

Dextran: Polydextran-based packing material primarily used for low pressure biochromatography; an example would be Sephadex (Amersham Pharmacia Biotech).

Dialysis: Dialysis works on the principles of the diffusion of solutes and ultrafiltration of fluid across a semipermeable membrane. Diffusion is a property of substances in water; substances in water tend to move from an area of high concentration to an area of low concentration. A semipermeable membrane is a thin layer of material that contains holes of various sizes, or pores. Smaller solutes and fluid pass through the membrane, but the membrane blocks the passage of larger substances (for example, red blood cells, large proteins). It is a technique used in biological sample prep to desalt biological fluids.

Diatomaceous earth: Also known as *diatomite* or ***kieselguhr,*** it is a naturally occurring, soft, siliceous sedimentary rock that is easily crumbled into a fine white to off-white powder. Diatomaceous earth consists of fossilized remains of diatoms, a type of hard-shelled algae. It has a particle size ranging from less than 3 µm to more than 1 mm, but typically 10–200 µm. Depending on the granularity, this powder can have an abrasive feel, similar to pumice powder, and is very light as a result of its high porosity. The typical chemical composition of oven-dried diatomaceous earth is 80–90% silica, with 2–4% alumina (attributed mostly to clay minerals) and 0.5–2% iron oxide; highly purified diatomaceous earth is used as a support for chromatography and for supported liquid–liquid extraction.

Diethylaminoethyl (DEAE): A popular weak anion-exchange functionality typically attached to cellulose or Sepharose (GE Healthcare); used for the separation of biomolecules.

Diethylene glycol succinate (DEGS): A GC stationary phase.

Diffusion coefficient (D_M or D_G; D_S or D_L): A fundamental parameter of a molecule in the liquid or gaseous mobile phase (D_M or D_G) or in the liquid stationary phase (D_S or D_L) that expresses the degree of free mobility of the molecule in solution. Expressed

in cm^2/s, the diffusion coefficient is dependent on molecular weight of the solute, temperature, solvent viscosity, and molar volume of the solute. A typical value of a small molecule (100 Da) in a liquid phase at room temperature is on the order of 10^{-5} cm^2/s. Gaseous solutes in helium carrier gas at 120 °C have diffusion coefficients that are several orders of magnitude higher, around 0.4 cm^2/s.

Digestion: The process of treating an insoluble chemical compound with a reactive substance (for example, for inorganic compounds it might be a strong acid; for a biological compound it might be an enzyme) that will break it down or disintegrate the compound into a more soluble form that can be further treated or analyzed.

Digital chromatography: The process of solid-phase extraction (SPE) is sometimes referred to as digital chromatography. A substance is either on the SPE stationary phase or is off the stationary phase during its retention and elution; in chromatography we are often trying to resolve closely related substances by exploiting subtle differences in retention in more of an analog separation mode; in terms of k values, ideally the solute in SPE has a value of infinity when on the sorbent and zero when eluted into solution.

Dilute and shoot: A simple sample preparation procedure where one merely dilutes the sample with solvent, mobile phase, or a compatible liquid and then injects that diluted sample into a chromatograph without any further sample preparation.

Dilution: Reducing the concentration of a chemical by adding an inert substance; the substance can be a liquid, solid, or gas.

Dimethylchlorosilane (DMCS): Sometimes used for silanizing glass GC parts such as inlet liners and endcapping silica-based HPLC bonded phases. Disposable presilanized inlet liners are a preferable alternative that avoid use and storage of this hazardous reagent.

Diode-array detection (DAD): Each wavelength of the UV and visible range of the light is measured with an individual diode. The optical resolution of the detector is defined by the number of diodes used (for example, 844 diodes: optical resolution = 1.4 nm).

Diol phase: A hydrophilic phase useful in both normal and reversed phase. It consists of a diol structure (two -OH groups on adjacent carbon atoms in an aliphatic chain). In normal-phase work, it is less polar than silica and in reversed phase work has been used for the separation of proteins and polypeptides.

Direct injection: Sample enters the inlet and is swept into the column by carrier gas flow. No sample splitting or venting occurs during or after the injection.

Direct sampling: A method of sample collection where a sample is taken directly from the source. For example, a cannister may be used to collect a gas sample exactly where the scientist desires. A river water sample can be obtained by lowering a collection vessel directly into the water. A thermal desorption tube can be used to concentrate volatile and semivolatile analytes by passing a gas stream through the adsorbent contained within the tube. This would be an example of direct sampling.

Direct thermal sampling: Refers to the process of using temperature as a variable in sample volatile and semivolatile substances; static headspace at

a given temperature is an example of thermal sampling; by selecting a certain temperature certain sample components can be ruled out because they may have extremely low volatility at a selected temperature; thermal sampling can occur in stages all the way up to pyrolysis where chemical bonds in the sample are purposely broken to access the structure of the material.

Disk: A number of sample preparation and separation media take the form of a disk; the most popular disks are used in filtration and may consist of any number of porous polymeric materials; the most popular types of SPE disks would have embedded particles in a disk made of PTFE or other inert polymeric material or a fiberglass matrix with interdispersed sorbent particles; some biological purification media employ the disk format. The stationary phases may contain ion exchange groups or other functionality that attracts solutes of interest or impurity that one may want to get rid of.

Disk cartridge (SPE): See ***disk***.

Dispersion: See ***system dispersion***.

Dispersive liquid–liquid microextraction (DLLME): The technique is based on a three-component solvent system. The container is usually a centrifuge tube and the appropriate mixture of immiscible organic extraction solvent (usually a few microliters, such as 8 µL of tetrachloroethylene) and a dispersive solvent (for example, ~1 mL of acetone) is rapidly injected with a syringe into an aqueous solution (~5 mL) containing the analyte of interest. When the three solvents are rapidly mixed, a cloudy solution is formed consisting of droplets of extraction solvent; the entire mixture is centrifuged and the droplet of solvent containing extracted analytes (tetrachloroethylene) is removed by a microsyringe for direct injection. Extraction is almost instantaneous and enrichment values are quite high.

Dispersive SPE (dSPE): In dSPE, loose SPE packing material is added directly to a solution rather than passing it through the packed material in a cartridge or tube; dSPE is most often used as the second step in QuEChERS where matrix compounds are removed from the organic solvent salting out extraction of the first step.

Displacement chromatography: Chromatographic process where the sample is placed onto the head of the column and then is displaced by a compound that is more strongly sorbed than the compounds of the original mixture. Sample molecules are then displaced by each other and by the more strongly sorbed compound. The result is that the eluted sample solute zones may be sharpened; displacement techniques have been used mainly in preparative HPLC applications.

Disposable filter: See ***syringe filter***.

Dissolution: The process of having a sample dissolve in an appropriate solvent.

Distillation: A method of separating mixtures based on differences in volatility of components in a boiling liquid mixture. Distillation is a unit operation, or a physical separation process, and not a chemical reaction; it can be used to purify organic compounds or to remove solvent; fractional distillation is used to separate compounds with close boiling points; azeotropic distillation is using an azeotrope to remove a solvent that has a boiling point too close or equal to another compound that cannot be separated.

Distribution constant (coefficient) (K_c): The equilibrium concentration of a component in or on the stationary phase divided by the equilibrium concentration of the component in the mobile phase; also called the *distribution coefficient* or in partition chromatography the *partition coefficient*; in partition chromatography K_c is used and refers to the case where the concentration in the stationary phase is expressed per unit volume of the phase: $V_R = V_M + K_c V_S$; in the case of a solid stationary phase, K_g is used and is expressed as per mass (weight) of the dry solid phase; in the case of adsorption chromatography with a well characterized adsorbent of known surface area, the concentration in the stationary phase is expressed as per unit surface area.

Dividers: A mechanical device used in subdividing solid powder samples into smaller units; they can be manual or automated; sample dividers will subdivide material samples into two smaller portions by a single pass or further subdivisions can be attained by multiple passes. The important feature of sample dividers is that each subdivision retains the characteristics of the original sample.

Dried blood spot (DBS) analysis: A newer method for sampling and transporting blood samples; a small (~15 µL) sample of whole blood is placed on a cellulose or other paper-like material and is dried for 2 h; the dried blood spot can be extracted to remove analytes of interest for further workup; has potential to replace drawing large quantities for blood analysis; used in conjunction with LC–MS-MS for high sensitivity and specificity.

Dried media spot (DMS) analysis: In addition to DBS, other biological fluids (for example, plasma, serum, cerebrospinal fluid, saliva) as well as other non-biological media have been investigated.

Drying: Drying of sample extracts can be accomplished by heating (evaporation), vacuum dessication, and other means; water can be removed (dried) from organic solvents by using anhydrous sodium sulfate.

Dwell time: The time equivalent to dwell volume; determined by the product of flow rate times the dwell volume.

Dwell volume: In LC, refers to the volume between the point of mixing of solvents (usually in the mixing chamber or at the proportioning valves in the liquid chromatograph) and the head of the chromatographic column; important in gradient elution or when changes in solvent composition are made in isocratic elution so that the column experiences the composition change in the shortest possible time. Low-pressure mixing systems generally have larger dwell volumes than high-pressure mixing systems.

Dynamic coating: The formation of in-situ coatings on the packing in HPLC or on capillary walls in CE by addition of a substance to the mobile phase that adsorbs (or absorbs) onto the packing or at the wall surface; the purpose of a dynamic coating is to generate a new stationary phase or to deactivate the packing material or capillary wall to prevent unwanted interactions; one simple example is the adjustment of the mobile phase or running buffer to a pH < 3 to protonate silanols and negate their effect; another is coating the phase with a hydrophilic polymeric material to prevent adsorption of proteins. In GC, dynamic coating applies a controlled-thickness coating to the inside of open-tubular columns as a solution flows through the column and leaves a thin coating behind, which is then

evaporated to yield a thin coating of any non-volatile material from the solution.

Dynamic headspace: See ***purge-and-trap sampling.***

E

Eddy dispersion (diffusion) term (*A* term): The *A* term in the van Deemter equation. It is the contribution to plate height that arises from the heterogeneity in axial velocities as a result of the particle size and geometry of the packing as well as wall effects: $a = 2\lambda d_p$. Typical values of λ for well-packed columns are 0.8–1.0. Some theories of chromatography indicate a velocity dependent contribution to the HETP from this process. Also known as *eddy diffusion*, *flow-heterogeneity induced broadening*, and *the multipath term*. The *A* term is zero in open-tubular GC columns. See ***van Deemter equation.***

Efficiency: The ability of a column to produce sharp, well-defined peaks. More efficient columns have more theoretical plates (*N*) and smaller theoretical plate heights (*H*). For asymmetric peaks it is better determined from the peak centroid and variance by mathematical analysis of the peak shape. See ***Foley-Dorsey equation.***

Effluent: The mobile phase leaving the column; the same as eluate.

Electrochemical detector: Global term for all detection modes recording electrical potential or current (conductivity, amperometric, pulsed amperometric, coulometric, and potentiometric detection). More often used for amperometric, pulsed amperometric, and coulometric detection only.

Electrochemical suppression: Continuously working chemical suppressor where H^+ or OH^- are electrochemically produced by the electrolysis of water. The expression is misleading as the suppression is chemical. Only the supply of the respective ion is done electrochemically.

Electrodialysis: Used to transport salt ions from one solution through ion-exchange membranes to another solution under the influence of an applied electric potential difference. This is done in a configuration called an electrodialysis cell. The cell consists of a feed (diluate) compartment and a concentrate (brine) compartment formed by an anion-exchange membrane and a cation-exchange membrane placed between two electrodes; can provide good enrichment factors.

Electrolytic suppression: Synonym to ***electrochemical suppression.***

Electrolytic conductivity detection (ELCD): An electrolytic conductivity detector catalytically reacts halogen-containing solute with hydrogen (reductive mode) to produce strong acid by-products that are dissolved in a working fluid. The acids dissociate and the increased electrolytic conductivity is measured. Other operating modes modify the chemistry for response to nitrogen- or sulfur-containing substances.

Electron-capture detection (ECD): An electron-capture detector ionizes solutes by collision with metastable carrier gas molecules produced by β-emission from a radioactive source such as ^{63}Ni. ECD is one of the most sensitive detection methods and responds strongly to halogenated solutes and others with a high electron-capture cross-section.

Electronic or programmed pressure control (EPC or PPC): Any of a number of pressure and flow control devices that incorporate electronic pressure or flow sensing and can be programmed from the GC microcontroller. Such devices enable method control of flow, velocity,

and pressure for GC columns, as well as providing a convenient means of incorporating gas-related parameters into electronic methods.

Eluate: Combination of mobile phase and solute exiting the column; also called ***effluent.***

Eluent: The mobile phase used to carry out a separation.

Eluite: The species being eluted: the analyte, or the sample.

Eluotropic series: A series of solvents (eluents) with an increasing degree of solvent strength generally used in liquid–solid or adsorption chromatography. In normal-phase chromatography, a nonpolar solvent such as pentane would be at one end of the scale, an intermediate solvent such as dichloromethane would be in the middle of the scale, and a strongly polar solvent such as methanol would be near the upper end of the scale. In normal-phase chromatography, the reverse order of strength would be observed; water would be weak and hexane strong. Thus, when developing a method or running a gradient, an eluotropic series is useful for selecting solvents.

Elute: To chromatograph by elution chromatography. The term *elute* is preferred to the term *develop* used in older nomenclature.

Elution: The process of passing mobile phase through the column to transport solutes down the column.

Elution chromatography: The most commonly used chromatographic method where the sample is applied to the head of the column as a narrow zone and individual molecules are separated and eluted at the end of the column under the influence of a directed flow of mobile phase. Compare to ***displacement chromatography*** and ***frontal analysis.***

Elution step: This is generally considered to be the fourth step in SPE and occurs after the washing (rinsing) step; in the elution step, analytes are removed from the SPE stationary phase by elution with a strong solvent so that the analytes are now in a concentrated state; often, the strong solvent is removed by evaporation and reconstituted in a solvent more compatible with the chromatographic system.

Elution volume (V_R): Refers to the volume of mobile phase required to elute a solute from the column. For a symmetric peak, it is the volume from the point of injection to the volume at maximum concentration (apex): $V_R = Ft_R$ where F is the flow rate and t_R is the retention time of the peak of interest.

Elutriation: A technique used to fractionate packing particles by size based on the difference in their Stokes terminal velocities. It is most often used for the separation of ion-exchange resins that need to have a particularly narrow size range, such as amino acid resins. The technique involves the upward flow of water into a large tube. The unsized beads are added to the moving water and the particles seek their own level, depending on their density and particle size. They are then removed at certain levels in the tube. High purity spherical silica gels are sometimes sized by elutriation.

Emulsion: A mixture of two or more liquids that are normally immiscible (nonmixable or unblendable). Emulsion is usually referred to when both the dispersed and the continuous phase are liquids. In an emulsion, one liquid (the dispersed phase) is dispersed in the other (the continuous phase). Emulsions are bothersome in LLE because they

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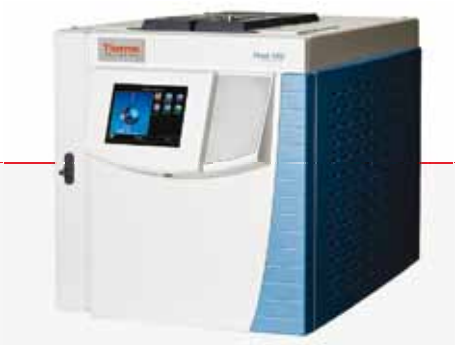

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sometimes are hard to break so that the layers can be separated and further treated, if necessary. There are numerous ways to break emulsions.

Enantiomeric compound: Chemical compounds that display chiral activity; such compounds will require a separation mechanism that can differentiate between the *R*- or *S*-enantiomer. Specialty columns are available for this purpose.

Endcapping: A technique used to remove silanol groups on silica gel that may remain after reaction with a large silylating agent such as octadecyltrichlorosilane. The column is said to be endcapped when a small silylating reagent (for example, trimethylchlorosilane and dichlorodimethylsilane) is used to bond residual silanol groups on a silica gel-based packing surface. Most often used with reversed-phase packings to minimize undesirable adsorption of basic, ionizable, and ionic compounds. For polymeric phases with terminal silanol groups, endcapping reactions are also used to remove them.

Endfitting: The fitting at the end of the column that permits connection to the injector or detector. Most HPLC endfittings contain a frit to hold the packing and have a low dead volume for minimum band spreading. They are usually constructed of stainless steel but PEEK and other polymeric materials are also used.

Equilibration: See ***column equilibration.***

Equivalent conductivity: The equivalent conductivity is a function of the concentration and the limiting conductivity (equivalent conductivity at infinite dilution).

Evaporation: The process of removing a volatile compound for the purposes of isolating a compound of interest; solvent evaporation is the most often used sample preparation technique.

Evaporative light scattering detection (ELSD): The effluent from the LC column is nebulized and then vaporized in a heated drift tube. This process results in a cloud of analyte particles that passes through a beam of light; the particles scatter the light and generate a signal at a photodiode or photomultiplier; it is a universal detection method where molecules are not required to have a chromophore, be fluorescent, or be electrochemically active.

Exchange capacity: See ***ion-exchange capacity.***

Excluded volume: See ***interstitial volume.***

Exclusion chromatography: See ***steric-exclusion chromatography*** and ***ion exclusion.***

Exclusion limit: In SEC, the upper limit of molecular weight (or size) beyond which molecules will be eluted at the same retention volume, called the ***exclusion volume.*** Many SEC packings are referred to by their exclusion limit. For example, a 10^5 column of porous silica gel will exclude any compounds with a molecular weight over 100,000, based on a polystryene calibration standard.

Exclusion volume (V_0): The minimum retention volume of a molecule on an SEC packing where all molecules larger than the size of the largest pore are totally excluded. These molecules are incapable of penetrating the pores and elute at the interstitial (interparticle) volume of the column.

Exponentially modified Gaussian peak: A theoretical model for peak asymmetry arising from the column, inlet, and detector. The basis for the Foley-Dorsey equations.

External calibration: Calibration mode using one or more standard solutions to establish the calibration function (calibration curve). With this function the concentration is calculated out of the measured peak area (or height) of the sample chromatogram. Linear or quadratic regressions are mainly applied but other regression modes are possible as well (for example, polynomial, point-to-point).

Extracolumn effects: The total band-broadening effects of all parts of the chromatographic system outside of the column itself. Extracolumn effects must be minimized to maintain the efficiency of the column. Areas of band broadening can include the injector, injection volume, connecting tubing, endfittings, frits, detector cell volume, and internal detector tubing. The variances of all of these contributions are additive.

Extracolumn volume: The volume between the effective injection point and the effective detection point, excluding the part of the column containing the stationary phase. It is composed of the volumes of the injector, connecting lines and frits, and the detector; it determines the extra column effects. See ***dead volume.***

Extraction: The general term for removing analytes of interest from a matrix.

F

FAME: Fatty acid methyl ester.

Fast GC: Gas chromatography performed on short, narrow-bore open-tubular columns, or on conventional columns at elevated linear velocities.

Fast LC: The use in HPLC of short columns (1.5–7 cm in length) with conventional internal diameters (2–6 mm) packed with small particles (d_p = 1.5–5 µm). Separation times in the range of minutes, sometimes seconds, are common; sometimes referred to as *ultrafast LC*.

Fast protein liquid chromatography (FPLC): A term coined to cover the specific use of HPLC for the separation of proteins. Generally, glass columns, moderate pressure, and spherical microbeads are used for FPLC.

FFAP: Free fatty-acid phase.

Fiberglass disks: A format where SPE particles are embedded in a fiberglass matrix; the disk format is especially useful for processing large volumes of sample (for example, water) given that the larger cross-sectional area allows for a higher flow rate than can be used for a typical cartridge.

Filter funnel: A filter funnel is a laboratory funnel used for separating solids from liquids via the laboratory process of filtering. To achieve this, a disk-shaped piece of filter paper is usually folded into a cone and placed within the funnel. The suspension of solid and liquid is then poured through the funnel. The solid particles are too large to pass through the filter paper and are left on the paper, while the much smaller liquid molecules pass through the paper to a vessel positioned below the funnel, producing a filtrate. The filter paper is used only once. If only the liquid is of interest, the paper is discarded; if the suspension is of interest, both the solid residue on the paper and the filtrate are kept for further analysis.

Filter holder: Membrane filters or membrane disks are sometimes furnished loose and can be placed in a holder (usually of stainless steel construction) for processing samples; after the filter is used the holder is opened

and the used filter is replaced with a fresh one.

Filter porosity: Pore size relates to the filter's ability to filter out particles of a certain size. For example, a 0.5-µm membrane will filter out particles with a diameter of 0.5 µm or larger from a filtration stream. Filter porosity is typically not related to, nor controlled by, pore size. These two parameters are essentially independent. Porosity is also unrelated to thickness. Rather, it is a function of the polymer and casting process used in the manufacture of the filter.

Filter vial: A filter vial is a membrane filter unit that consists of two pieces. One is the filtration plunger, which contains a membrane filter suitable for the solvent being filtered. The second part of the filter vial is the vial body itself; once the sample is loaded and filtered, the filter vial can be placed directly in the autosampler without transferring the filtered sample to another vial.

Filtration: The process of passing a liquid through a paper, membrane, glass, or other type of filter for the purposes of removing particulates that could cause problems downstream during a chromatographic analysis; a chemical filter also removes certain chemical species. See ***chemical filtration.***

Fixed-well plate: A 96-well plate with fixed (nonremovable) wells, not an array. See ***96-well plate*** and ***array 96-well plate***.

Flame ionization detection (FID): A flame ionization detector ionizes hydrocarbon-containing solutes in a hydrogen–air flame. FID is a nearly universal detection method that responds strongly to most classes of organic compounds.

Flame-photometric detection (FPD): A flame-photometric detector burns heteroatomic solutes in a hydrogen-air flame. The visible-range atomic emission spectrum is filtered through an interference filter and detected with a photomultiplier tube. Different interference filters may be selected for sulfur, tin, or phosphorus emission lines. FPD is a sensitive and selective detection method.

Flash chromatography: A very fast form of classical liquid chromatography used by synthetic organic chemists to effect rapid purification. Done primarily in the normal-phase mode, sometimes with reversed-phase LC. See ***column chromatography.***

Flexible well plate: See ***array 96-well plate.***

Flow injection extraction: An on-line extraction technique where a sample is injected as a plug into an aqueous flow stream that is divided into small segments by an organic phase; the aqueous and organic segments are mixed during their passage down a coil, and eventually the phases are separated at the end by a special fitting and the amount of extract compound can be measured in the organic phase.

Flow programming: Used to decrease the retention time of slow-moving compounds. Flow programming is occasionally used in concert with temperature programming in GC.

Flow rate (F, F_c): The volumetric rate of flow of mobile phase through a column. For a conventional 4.6-mm i.d. HPLC column, typical flow rates are 1–2 mL/min. GC packed column flow rates typically range from 10 to 40 mL/min, and open-tubular column flows range from less than 1 mL/min up to about 10 mL/min.

Flow resistance parameter (Φ): $\Phi = d_p^2/B_o$ See ***permeability***.

Fluoro phase: One of a family of aliphatic and aromatic reversed-phase materials in which a substantial fraction of the bonded phase is fluorinated. Sometimes called *fluorous phases* or *perfluoro phases*. Typically have different selectivities than hydrocarbon phases.

Fluted filter paper: Filter paper that is folded in a systematic manner to allow more air space in the filter funnel; this allows liquid to flow faster through the filter paper.

Foley-Dorsey equation: A correction of the plate count and retention time to correct for peak tailing from extracolumn sources of broadening. (See J.P. Foley and J.G. Dorsey, *Anal. Chem.* **55,** 730–737 [1983].)

Forced-flow leaching: Solid material is packed into stainless steel column and toluene (or other extraction solvent) is pumped into the column under pressure and with heating; hot solvent leaches (extracts) out extractable compounds which are collected at the exit of the column.

Fraction of analyte extraction (*E*): The fraction of analyte extracted: $E = (C_o V_o)/(C_o V_o + C_{aq} V_{aq}) = (K_D V)/(1 + K_D V)$ where V_o is the volume of organic phase, V_{aq} the volume of aqueous phase, and V is the phase ratio V_o/V_{aq}.

Fractionation range: In SEC, refers to the operating range of a gel or packing. This is the range in which the packing can separate molecules based on their size. At one end of the range, molecules that are too large to diffuse into the pores are excluded. At the other end of the range, molecules that can diffuse into all of the pores totally permeate the packing are eluted (unseparated) at the permeation volume.

Freeze drying: The process of removing water, mainly from biological samples, by using vacuum sublimation.

Frictional heating: Viscous heating of solvent molecules passing through very small-diameter (micrometer) particles; causes a rise in temperature over the length of the column; reduced by using smaller-diameter columns (smaller heat dissipation) and superficially porous particles, which also have improved heat dissipation.

Frit: The porous element at either end of a column that serves to contain the column or SPE packing. It is placed at the very ends of the column tube or in the end fitting. Frits can be stainless steel or another inert metal or plastic such as porous PTFE or polypropylene. The frit porosity must be less than the smallest particle in the column; otherwise particles will pass through the frit and packing will be lost.

Frontal analysis: A chromatographic technique that involves continuous addition of sample to the column with the result that only the least sorbed compound, which moves at the fastest rate, is obtained in a pure state. The second-least-sorbed compound is eluted with first eluted compound, the third-least-sorbed compound with the first and second compound, and so forth until the original sample passes through the column exit. Frontal analysis is seldom used and is mainly a preparative technique.

Frontal chromatography: The same as ***frontal analysis***.

Fronting: Peak shape in which the front part of the peak (before the apex) in a chromatogram tapers in advance of the remainder of the peak (that is, the front is less steep than the rear). There is an asymmetric distribution with a leading

edge. The asymmetry factor for a fronting peak has a value less than 1. The opposite effect is tailing. Fronting can result at high sample loads because of positive curvature in the isotherm and from use of poorly packed columns. See ***asymmetry factor.***

Fused-core packing: See ***superficially porous particles (SPPs).***

Fused silica: Open-tubular GC columns and LC nanocolumns are commonly manufactured from fused-silica tubing that is coated externally with a protective polymeric material such as polyimide.

Fused-silica open-tubular column (FSOT): Open-tubular GC columns made of fused silica. See ***open-tubular column.***

G

Gas–liquid (phase) chromatography (GLC, GLPC): Solutes partition between a gaseous mobile phase and a liquid stationary phase. Selective interactions between the solutes and the liquid phase give rise to different retention times in the column.

Gas-phase extraction: See ***direct thermal sampling.***

Gas–solid chromatography (GSC): Solutes partition between a mobile gaseous phase and a solid stationary phase. Selective interactions between the solutes and the solid phase give rise to different retention times in the column.

Gaussian curve: A standard error curve, based on a mathematical function, that is a symmetrical, bell-shaped band or peak. Most chromatographic theory assumes a Gaussian peak. Use of the peak maximum position as a measure of retention and equations mentioned above for efficiency assume a Gaussian peak shape.

Gaussian peak: The equation of a Gaussian distribution can be written in terms of chromatography parameters as $C = C_{max}\exp(-(t - t_R)^2/2\sigma^2)$

Gel: The solid packing used in gel chromatography or GPC. An actual gel consists of two parts: the dispersed medium (solid portion) and the dispersing medium (the solvent). Also defined as a colloidal dispersion of a solid and liquid in which the solid is the continuous phase.

Gel filtration chromatography (GFC): Also called *aqueous size-exclusion chromatography*; carried out with aqueous mobile phases. Generally refers to molecular size separations performance on soft gels such as polydextrans but highly crosslinked polymers, silica gels, and other porous media may also be used. Most gel filtration separations involve biopolymers and water-soluble polymers such as polyacrylic acid.

Gel permeation chromatography (GPC): Size-exclusion chromatography (SEC) carried out with organic mobile phases used for the separation and characterization of polymers. SEC with aqueous mobile phases is referred to as ***gel filtration chromatography.***

Ghost peaks: Peaks not present in the original sample. Ghost peaks can be caused by septum bleed, solute decomposition, or carrier-gas contamination.

Gigapores: See ***perfusion chromatography.***

Golay equation: M.J.E Golay formulated an equation for the theoretical plate height versus the average linear velocity of open-tubular (capillary) columns. The Golay equation is similar to the van Deemter equation, except that the *A* term is dropped because there is no column packing, and the *B* and *C*

terms are modified accordingly as well: $h = (B/\overline{u}) + \overline{u}(C_M + C_S)$

Grab sampling: In gas sampling, an evacuated sample canister can be opened and sample rapidly collected at an uncontrolled rate, usually over several seconds, until the container reaches equilibrium with atmospheric pressure. Generally this qualitative approach is used when unknown analytes must be identified, when the air contains high concentrations of analytes at certain (short) times, or when an odor is noticed and a sample must be obtained quickly. Paired grab samples (before–after or smell–no smell) often are used to qualitatively diagnose a perceived problem.

Gradient: A process to change solvent strength as a function of time (normally solvent strength increases) thereby eluting progressively more highly retained analytes; typically gradients can be binary, ternary, and quaternary solvent mixtures where solvents are blended to achieve the proper strength. In GC, an older term that refers to column temperature programming to achieve a similar effect.

Gradient delay volume: See ***dwell volume.***

Gradient elution: Technique for decreasing separation time by increasing the mobile-phase strength over time during the chromatographic separation. Also known as *solvent programming*. Gradients can be continuous or stepwise. Binary, ternary, and quaternary solvent gradients have been used routinely in HPLC.

Graphitized carbon: Graphitized carbon is a graphitic carbon with more or less perfect three-dimensional hexagonal crystalline order prepared from non-graphitic carbon by graphitization heat treatment; this packing material has a strong affinity for polar compounds in aqueous samples and water miscible organic extracts. Commonly used in pesticide analysis of food samples; also known as *graphitized carbon black (GCB)*. Also used as a GC stationary phase.

Graphitized carbon packing: A reversed-phase packing material which consists of pure graphitic carbon; possesses interesting sorbent properties such as preferential separation of geometric isomers such as *o*-, *m*- and *p*-aromatics and *cis*–*trans* isomers.

Grinding: Both manual and automated mortar and pestles are available; grinding can be performed under wet or dry conditions; by this process particles of approximately 10 µm can be achieved.

Guard column: A small column placed between the injector and the analytical column. It protects the analytical column against contamination by sample particulates and strongly retained species. The guard column is usually packed with the same material as is in the analytical column and is often of the same inner diameter. Its length is much shorter, it costs less, and is usually discarded when it becomes contaminated. Integrated guard–analytical column systems are often preferred to minimize extracolumn effects caused by the use of connecting tubing with separate guard and analytical columns. For GC, see also ***retention gap.***

H

Headspace sampling: Gas-phase sampling technique in which solute is removed from an enclosed space above a solid or liquid sample. The headspace refers to the vapors that form above liquids and solids; if the sample is in

thermodynamic equilibrium with the gas phase in a closed thermostated vessel, the method is called *static headspace sampling*; if an inert gas passes over or through the sample and stripped sample volatiles accumulate in an adsorbent or cryogenic trap, the method is *dynamic headspace* or *purge-and-trap sampling*.

Headspace single-drop microextraction (HS-SDME): A single drop of solvent (1–2 µL) suspended in the headspace can partition volatile analytes into the solvent; the drop can be withdrawn into the syringe and injected into a GC system.

Headspace solid-phase microextraction (HS-SPME): Instead of a drop of solvent, a polymer-coated fiber can be placed in the headspace and once the analytes adsorb on the polymer coat, the fiber can be transferred to a GC inlet and the sorbed analytes volatilized by thermal desorption.

Heart cutting: In preparative LC, refers to collection of the center of the peak where purity should be maximum. The term is also used for analytical column switching for LC or GC in which two or more partially resolved peaks eluted from one column are directed onto another column of different polarity, or at a different temperature, for improved resolution.

Helium ionization detection (HID): An ionization detection method for GC. Helium is ionized by a radioactive or other energetic source; the resulting He^{2+} ions interact with solutes to produce ions that measured with a sensitive electrometer.

Helium plasma or microwave-induced detection (HPD): A helium plasma is created in a microwave RF field. The plasma emits UV radiation that ionizes solute molecules, which are measured with an electrometer. Metastable He may also be harnessed in an electron-capture mode for halogen-specific response. See also ***pulsed discharge detection.***

Helium sparging: See ***degassing.*** Helium has a very low solubility in most common liquids.

High performance capillary electrophoresis (HPCE): A technique where small diameter capillaries, buffered conducting solutions, and high voltage (up to 30,000 V) are used to separate ionic molecules based on their differential electrophoretic mobilities; nonionic (neutral) molecules can be separated by MEKC.

High performance liquid chromatography (HPLC): Modern liquid-phase chromatography technique using small particles and high pressures.

High-pressure mixing: A configuration of a gradient HPLC system where the solvents are mixed on the high-pressure side of multiple pumps (usually two, binary); such a system offers a lower gradient delay volume than low pressure mixing systems where the solvents are mixed by proportioning valves before a single pump.

High-abundance protein depletion: By using antibody columns specific for the highest abundance proteins, they can be selectively removed from plasma. This process enables investigation of the lower abundance proteins, which may be biomarkers or other compounds of interest.

Hold-up time (t_M, t_0): The time required for an unretained compound to be eluted, or the time required for one column volume (V_M) of mobile phase to pass through the column. In reversed-phase LC, uracil is often used

to measure hold-up volume and hold-up times. In GC, methane or another unretained compound is used to measured the column hold-up time. Also called the *unretained peak time* or *dead time*.

Hold-up volume (V_M or V_0): The total volume of mobile phase in the column regardless of where it exists. In LC: The hold-up volume consists of the entire space accessible to a small molecule able to fully permeate all the pores of the packing material. It comprises the interstitial volume and the unoccupied pore volume. It is denoted as V_o or V_M. The system hold-up volume includes the additional volume in the tubing used to connect the injector and detector to the column. The hold-up volume is usually approximated by injecting a small essentially unretained species. In reversed-phase LC, uracil, acetone, and thiourea are most commonly used: $V_M = t_M F_c$. In GC: The gas-phase volume of the column that corresponds to the hold-up time. Measured by injecting an unretained species such as methane that fits in all the pores. See ***hold-up time.***

Hold-up volume, corrected (V_M^0): The hold-up volume corrected for carrier-gas expansion along the column: $V_M^0 = Vj$

Hollow-fiber liquid-phase microextraction (HF-LPME): A hollow-fiber (HF) membrane technique where an HF membrane separates two extraction phases; the membrane serves as a barrier and can be impregnated with solvent to permit liquid–liquid or liquid–liquid–liquid extractions to take place; the membrane can be selected to allow certain analytes to pass through but not others.

Homogenization: The process of making a sample more uniform by size reduction and blending; homogenizers with high speed blades are available to do the job.

Hybrid silica: Silica gel consisting of both organic and inorganic moieties with hybrid properties of polymeric packings and silica packings; synthesized from silanes containing organic functionality; different selectivity but higher high pH stability than bare or uncoated silica gel.

Hydrodynamic volume: The molecular volume defined by the effective diameter of a molecule in free solution where the hydrodynamic sphere would be a sphere defined by the molecule as it revolves around its central axis in solution; termed used in size-exclusion chromatography to define molecular shape and to explain why molecules with the same molecular weight often have totally different elution volumes; measured by determining the Stokes radius.

Hydrophilic: "Water loving"; refers both to stationary phases that are fully compatible with water or to water-soluble molecules in general. Many columns used to separate proteins (ion exchange, SEC, affinity) are hydrophilic in nature and should not irreversibly sorb or denature protein in the aqueous environment.

Hydrophilic interaction liquid chromatography (HILIC): An alternative technique to reversed-phase HPLC for the separation of highly polar analytes that may be only slightly retained or unretained by reversed-phase LC, HILIC requires a high percentage of a nonpolar mobile phase and a polar stationary phase, similar to the requirements in normal-phase

chromatography. However, unlike normal-phase chromatography, which uses nonpolar solvents such as hexane and methylene chloride and tries to exclude water from the mobile phase, HILIC requires some water in the mobile phase to maintain a stagnant enriched water layer on the surface into which analytes may selectively partition. In addition, water-miscible organic solvents are used instead of the water-immiscible solvents used in normal-phase chromatography. With HILIC, sorbents such as bare silica, bonded diol, and polyhydroxyethylaspartamide are used. Polar analytes are well retained and are eluted in order of increasing hydrophilicity, just the inverse of reversed-phase LC.

Hydrophobic: "Water hating"; refers both to stationary phases that are not compatible with water or to molecules in general that have little affinity for water. Hydrophobic molecules have few polar functional groups; most have a high content of hydrocarbon (aliphatic and aromatic) functionality.

Hydrophobic interaction chromatography (HIC): A technique in which weakly polar (nonhydrocarboneous) packings are used to separate molecules by virtue of the interactions of their hydrophobic moieties and the hydrophobic sites on the packing surface. High concentrations of salt solutions are used in the mobile phases and separations are affected by changing the salt concentration. The technique is analogous to "salting out" molecules from solution. Gradients are run by decreasing the salt concentration; often used for the separation of proteins that are sensitive to denaturization by the organic solvents used in regular reversed-phase chromatography; usually little or no organic solvent is used in the mobile phase in HIC.

Hydrophobic subtraction model: Developed by Lloyd Snyder and John Dolan, this model is used to characterize reversed-phase columns; using five types of probes, based on their equations, they can predict if a certain column will be close in selectivity characteristics to another column or totally different (orthogonal); over 300 columns have been characterized using this model. (See L. Snyder and J. Dolan, *LCGC* **20**[11], 1016–1026 [2002].)

Hydroxyapatite: A porous calcium hydroxyphosphate solid that chemically resembles bone and tooth used as a packing material used in biochromatography for nucleic acid constituents, monoclonal antibodies, and proteins.

Hypercrosslinking: Mainly refers to a new way to synthesize a polymeric monolith; hypercrosslinked monolithic capillary columns contain an array of small pores and have very high surface areas.

Hyphenated techniques: Refers to the family of techniques best known by their abbreviations, including LC–MS, LC–FTIR, and MS-MS.

Immobilized liquid extraction: Similar to SPE but a polymeric stationary phase is bonded to the inside of a glass vial; analytes partition into polymeric phase and loading, washing, and elution steps are performed by addition of various solvents.

Immobilized metal affinity chromatography (IMAC): See ***metal affinity chromatography.***

Impinger: Impingers are glass bubble tubes designed for the collection of airborne hazards into a liquid medium. When using a personal air sampler, a known volume of air bubbles is pumped through the glass tube that contains a liquid specified in the method. The liquid is then analyzed to determine airborne concentrations. An impinger may be mounted on the side of an air sample pump or put into a holster and placed near a worker's breathing zone.

Imprinted phases: Polymer and silica phases generated in the presence of a "template" or "printing" molecule. Such phases have enhanced selectivity for the templating molecule; also called ***molecularly imprinted phases (MIPs)***.

In-line filter: A device that prevents particulate matter from damaging the column. Modern low-volume, in-line filters can be placed between the injector and the column without major contributions to band broadening. A filter in this position is used to prevent sample particles from entering the packed bed or column inlet frit.

In situ derivatization: The act of derivatizing a compound of interest in its native environment; for example, for a tenaciously held analyte on a soil sample changing its chemical nature by performing an in situ derivatization, the compound may be more easily released and isolated for further workup or analysis; derivatization may also be formed in solution and the derivatized compound extracted by LLE.

Included volume: Also known as totally included volume. The volume at which a small molecule that explores the entire pore space of a column is eluted. See ***steric-exclusion chromatography.***

Indirect detection: Used for non-ultraviolet-absorbing or nonfluorescing analytes; a UV-absorbing or fluorescent compound is added to the mobile phase that maintains a high background signal; when a nonabsorbing or nonfluorescing analyte is eluted, the background is diluted and a negative peak is observed for that analyte; when an analyte acts to increase the concentration of the indicating species, a positive peak is observed. When a negative signal is detected, the detector signals are reversed to the output device.

Infinite diameter column effect (IDE): Name given by John Knox to the following phenomenon: At a certain column length, a sample injected into center of a packed bed spreads by radial diffusion but never reaches column wall, where wall effects can cause band broadening. Knox showed that a sample peak collected in the exact center of the column exit displayed a higher efficiency than a sample peak collected near the wall. The infinite diameter effect depends on column length, internal diameter, particle size, and mobile-phase properties. Very seldom applied in HPLC.

Injection solvent: Solvent used to inject sample into an HPLC column; solvent should be of equal or lower strength than the mobile phase to prevent premature movement down the column due to the presence of a stronger solvent.

Inlet: In LC, the initial part of the column where the solvent and sample enter. There is usually an inlet frit that holds the packing in place and, in some cases, protects the packed bed. In GC, a device between the carrier gas source and the column inlet that transfers sample from outside the chromatograph into the column, often by vaporizing the sample. See ***split injection, splitless***

injection, on-column injection, programmed temperature vaporizer.

Inlet filter: Filtration devices attached to the inlet lines of the pump that removes particulate matter from the mobile phase before the solvent reaches the pump; reservoir filters are an inlet filter that resides in the solvent bottle.

Inlet liner: Deactivated glass tube in an inlet system into which liquid sample is injected. An inlet liner may be open or packed with deactivated glass wool, and it may have various internal structures. The purpose is to vaporize and disperse evaporating sample into the carrier gas stream as uniformly as possible while not causing significant sample breakdown, adsorption, or discrimination.

Inlet/outlet check valves: The check valve (or valves) on an LC pump that allows mobile phase to flow in one direction but not in the reverse direction. The inlet check valve allows flow from the reservoir into the pump, and the outlet check valve allows mobile phase to flow to the column from the pump.

Instrumental bandwidth: The contribution of the analytical instrument to peak broadening; see ***extracolumn effects*** for explanation.

Instrumental dispersion: See ***extracolumn effects.***

Internal standard (IS): In quantitative analysis, precision and accuracy are greatly improved by use of internal standards (IS). The procedure involves the addition of a fixed amount of internal standard to a series of increasing concentrations of reference sample and to the unknown concentration. The ratio of the areas of the reference concentrations and the areas of the IS is plotted against the known concentration of the reference samples. The internal standard should be chemically similar to the substance being quantitatively determined and should have a retention time fairly close to it.

Interparticle porosity (ε): The interparticle volume of a packed column per unit column volume: $\varepsilon = V_o/V_c$. See also ***interstitial porosity.***

Interstitial porosity (ε_e): The fraction of the volume in the column located in the interparticle (interstitial) space: $\varepsilon_e = V_e/V_c$

Interstitial volume (V_e): The volume between the particles. Does not include the volume in the pores of the particles. Also called the excluded volume (see ***steric-exclusion chromatography***) and interparticle volume. Measured by injecting a molecule which does not permeate any pores and does not interact with the surface of the particles. In SEC this volume is denoted V_o.

Intraparticle porosity (ε_i): The fraction of the particle volume which lies in the pores: $\varepsilon_i = V_{pore}/V_{particle}$

Intraparticle volume (V): The volume inside the pores of the particles. Also called the *internal volume* and ***included volume***. Can be measured by BET or mercury intrusion porosimetry.

Ion chromatography (IC): An ion-exchange technique in which low concentrations of organic and inorganic anions or cations are determined using ion exchangers of low ion-exchange capacity with dilute buffers. Conductivity detectors are often used. Ion chromatography is practiced in two forms. In suppressed IC, a second column or a membrane separator is used to simultaneously remove the buffer counterion to the analyte and replace it with hydrogen or hydroxide ion, which concomitantly converts the buffer to an uncharged species, thereby supressing background and enhancing sensitivity. In nonsuppressed IC, weakly conducting buffers at low

concentration are carefully selected and the entire effluent is passed through the detector — ions are detected above the background signal.

Ion exclusion: The process in which ionized solutes can be separated from unionized or partially ionized solutes using ion-exchange resins. Separation results from Donnan potential where ionic solutes exist at a higher concentration in solution than in the stationary phase whereas nonionic solutes are evenly distributed between the mobile phase and resin. Therefore, ionic solutes will move faster down the column than non-ionic solutes. Ion exclusion is known to take place in reversed-phase LC when anions are separated at pH values where the silanol groups are ionized.

Ion moderated partitioning chromatography: A technique used for the separation of carbohydrates using strong cation-exchange packings that are in specific cationic form (for example, calcium, hydrogen, silver); the separation mechanism is complexation rather than ion exchange.

Ion retardation: Refers to the use of amphoteric ion-exchange resins, which are used to retard ionic molecules and allow nonionic molecules or nonelectrolytes to be eluted preferentially.

Ion suppression: Buffering in an aqueous mobile phase at a particular pH to suppress solute ionization. For example, weak carboxylic acids can have their ionization suppressed by the adjustment of the pH below their pK_a. Useful for improving peak shape of weak acids and bases in reversed-phase LC.

Ion-trap detector: Mass spectrometric detector that uses an ion-trap device to generate mass spectra.

Ion-exchange capacity: The number of ionic sites on the packing that can take place in the exchange process. The exchange capacity is expressed in millieqivalents per gram. A typical styrene-divinylbenzene strong anion exchange resin may have 3–5 meq/gm capacity. Exchangers for IC have very low capacity. Capacity of weak anion and cation exchangers varies dramatically with pH.

Ion-exchange chromatography: A mode of chromatography in which ionic substances are separated on cationic or anionic sites of the packing. The sample ion (and usually a counterion) will exchange with ions already on the ionogenic group of the packing. Retention is based on the affinity of different ions for the site and a number of other solution parameters (pH, ionic strength, counterion type, and so forth). Ion chromatography is basically an ion-exchange technique.

Ion-pair chromatography: Form of chromatography in which ions in solution can be "paired" or neutralized and separated as an ion pair on a reversed-phase column. Ion-pairing agents are usually ionic compounds that contain a hydrocarbon chain that imparts a certain hydrophobicity so that the ion pair can be retained on a reversed-phase column. Retention is proportional to the length of the hydrophobic chain and the concentration of the ion-pair additive. Ion pairing can also occur in normal-phase chromatography when one part of the pair is dynamically loaded onto a sorbent, but this technique is not as popular as reversed-phase LC. Also known as ion-interaction chromatography or dynamic ion-exchange chromatography, stressing the fact that the precise mechanistic details of how the additive controls retention are not always known.

Ionic strength: Ionic strength is a characteristic of an electrolyte solution.

It is typically expressed as the average electrostatic interactions among an electrolyte's ions. It is related to electrolyte concentration, but the main difference between ionic strength and electrolyte concentration is that the ionic strength is higher if some of the ions are more highly charged. The higher the ionic strength of a mobile phase the more the mobile phase competes with the analyte for ionic or adsorptive sites.

Irregular packing: Refers to the shape of a column packing. These irregular packings are available in microparticulate sizes. The packings are obtained from grinding solid materials into small particles and then sizing them into narrow fractions using classification machinery. Spherical packings are now used more than irregular packings in analytical HPLC but the less-expensive irregular packings are still widely used in preparative LC.

Irreversible adsorption: When a compound that has a very strong affinity for an adsorbent is injected onto a column, it can be adsorbed so strongly that it cannot be eluted from the column. A chemical reaction between the sample and the surface of the adsorbent is an example of irreversible adsorption.

Isocratic: Use of a time-invariant eluent composition in LC.

Isolate: Analyte to be isolated from matrix background and then analyzed.

Isotherm: See ***adsorption isotherm.***

Isothermal chromatography: Use of conditions of constant column temperature. The vast preponderance of all LC is done under isothermal conditions, while most GC separations are performed with column temperature programming.

Isotope-coded affinity tags (ICAT): Isotope-coded affinity tags (ICATs) are a gel-free method for quantitative proteomics that relies on chemical labeling reagents. These chemical probes consist of three general elements: a reactive group capable of labeling a defined amino acid side chain (for example, iodoacetamide to modify cysteine residues), an isotopically coded linker, and a tag (for example, biotin) for the affinity isolation of labeled proteins and peptides. For the quantitative comparison of two proteomes, one sample is labeled with the isotopically light (d_0) probe and the other with the isotopically heavy (d_8) version. To minimize error, both samples are then combined, digested with a protease (such as trypsin), and subjected to avidin affinity chromatography to isolate peptides labeled with isotope-coded tagging reagents. These peptides are then analyzed by liquid chromatography-mass spectrometry (LC–MS). The ratios of signal intensities of differentially mass-tagged peptide pairs are quantified to determine the relative levels of proteins in the two samples. The original tags were developed using deuterium, but later the same group redesigned the tags using ^{13}C instead to circumvent issues of peak separation during LC because of the deuterium interacting with the stationary phase of the column.

K

Kieselguhr: A diatomaceous earth used both in column chromatography and as a sample cleanup media. Only weakly adsorptive, it is also used as a support in liquid–liquid chromatography and in supported liquid extraction, see ***supported liquid extraction (SLE).*** Rarely used in HPLC.

Kilopascal (kPa): A unit of pressure. 100.0 kPa = 1.0 bar = 0.9869 atm. 101.325 kPa = 1.0 atm.

Kinetic plot: Kinetic plots are methods to characterize the practical limits of column performance, where theoretical plates (H) and separation impedance (E) are plotted as a function of the pressure-drop limited plate number (N). The kinetic plot retains the information shown in van Deemter plots but completes it with the information on the bed permeability. See ***Poppe plot.***

Knox equation: A modification of the van Deemter equation developed by John Knox that uses reduced terms, in which the A term that represents eddy dispersion is replaced with $Av^{1/3}$ where v is the reduced interstitial eluent velocity.

L

Laminar flow: The smooth time-invariant flow that develops when a liquid is moving under conditions where viscous forces dominant over inertial forces. Laminar flow is characterized by a low ***Reynolds number***. For flow in a cylindrical tube fluid stream-lines near the center move faster than those at the tube wall which are static resulting in a radially parabolic distribution in axial fluid velocity. This nonuniformity of axial velocities in the interstices in a packed bed also leads to substantial peak broadening in packed columns.

Langmuir adsorption isotherm: A specific form of an isotherm $C_S = (N_0 C_M)/(K_d + C_M)$ where C_S and C_M are the equilibrium stationary and mobile phase concentrations of the solute, N_0 the total number of surface sites available for sorption and K_d the sorption binding constant.

Large-volume injection (LVI): A technique for introduction of larger than normal volumes of sample in a solvent into a capillary GC column; in this approach, the bulk of the solvent is evaporated before the sample transfers to the analytical column; there are two popular LVI techniques: programmed temperature vaporization and cool on-column injection with solvent vapor exit; both are approaches to lowering detection limits.

Ligand: In ligand-exchange chromatography, refers to the analyte that undergoes ligand exchange with the stationary phase. In affinity chromatography, refers to the biospecific material (enzyme, antigen, or hormone) coupled to the support (carrier) to form the affinity column. In bonded-phase chromatography this term denotes the moiety covalently bound to the surface.

Ligand-exchange chromatography: A technique in which chelating ligands are added to the mobile phase and undergo sorption onto a packing. These sorbed molecules can act as chelating agents with certain solutes. An example would be the use of copper salt added to the mobile phase for the chelatation and separation of amino acids. Chelating resins function in a similar manner, where chelating groups are chemically bonded to the polystyrene backbone.

Limit of detection (LOD): The concentration of the analyte at which the resulting peak can be distinguished from baseline noise. Literature and norms describe different ways of determining the LOD.

Limit of quantitation (LOQ): The minimum concentration of the analyte at which the resulting peak can be quantified with a defined level of certainty. Typically 3–5 times higher than the LOD.

Linear elution adsorption chromatography (LEAC): A term coined by Lloyd Snyder; refers to adsorption chromatography carried out in the linear portion of an adsorption isotherm; sometimes referred to as *linear chromatography*.

Linear range (LR): Also, *linear dynamic range*. The range of solute concentration or amount over which detector response per solute amount is constant within a specified percentage.

Linearity: In quantitative analysis, it is essential for the detector to yield a linear response with respect to solute concentration; some detectors may show nonlinear performance in certain concentration ranges, especially on the high end but also on the low end.

Liquid chromatography: A separation technique in which the mobile phase is a liquid; most often carried out in a column.

Liquid phase: In GC, a stationary liquid layer coated on the inner column wall (WCOT) or on a support (packed, PLOT, SCOT) that selectively interacts with different solutes to produce different retention times. Also refers to the stationary phase in LLC.

Liquid–liquid chromatography (LLC): Same as partition chromatography. One of the earliest separation modes of HPLC; it gave way to chemically bonded phases in the early 1970s.

Liquid–liquid diffusion coefficient (D_L): See ***diffusion coefficient.***

Liquid–liquid extraction (LLE): LLE is an extraction technique for separating interferences from the analytes by partitioning the analytes between two immiscible liquids (or phases); one phase is usually aqueous and the second phase an organic solvent; more hydrophilic compounds prefer the aqueous phase while more hydrophobic compounds will be found in the organic solvent; by the use of additives (for example, buffers or ion-pair reagents), equilibria can be shifted to "force" analytes or matrix compounds into one or other of the two layers.

Liquid-phase microextraction (LPME): A liquid extraction technique where there is a great reduction in the acceptor-to-donor phase ratio; a hollow fiber is impregnated with an organic solvent used to accommodate or protect microvolumes of acceptor solution. This novel methodology proved to be an extremely simple, low-cost, and virtually solvent-free sample-preparation technique that provided a high degree of selectivity and enrichment by additionally eliminating the possibility of carryover between runs.

Liquid–solid chromatography (LSC): Same as ***adsorption chromatography.***

Liquid–solid extraction: The general expression for extraction techniques that uses an organic solvent to extract analytes from a solid material. In its simplest form, the "shake flask" extraction takes place at room temperature and works well for the case where the matrix is porous and the analytes are easily extractable.

Loadability: The maximum amount of analyte that can be injected onto a column above which it no longer permits the isolation of product at the desired level of purity or recovery level; important in preparative chromatography.

Loading (phase loading versus sample loading): The amount of stationary phase coated or bonded onto a solid support. In liquid–liquid chromatography, the mass of liquid phase per gram of packing. In bonded-phase chromatography, the loading may be expressed in μmol/m^2 or %C (w/w); also called coverage or surface coverage. An alternate

(unrelated) meaning is the amount of sample mass that is injected on an analytical or preparative column; preparative columns are often operated in an overloaded condition for throughput reasons.

Loading step (SPE): The second step in SPE (after conditioning) where the sample is loaded onto the SPE phase (cartridge).

Log K_W: In reversed-phase LC, the extrapolated intercept of a plot of log *k* versus volume fraction of organic modifier in reversed-phase LC. See also ***solvent strength (S)***.

Longitudinal diffusion: Same as molecular diffusion term; *B* term in van Deemter equation; see ***van Deemter equation.***

Low pressure mixing: See ***high pressure mixing.***

Lyophilization: The process of dehydrating a sample, often biological, containing water by using vacuum sublimation; also referred to as freeze drying.

M

Maceration: The process of breaking down a soft material into smaller parts by tearing, chopping, cutting, and so forth.

Macroporous resin (macroreticular): Cross-linked ion-exchange resins that have both micropores of molecular dimensions but also macropores of several hundred angstroms wide. These are highly porous resins with large internal surface area accessible to large molecules.

Magnetic-bead technology: Micro magnetic beads are uniform polymer particles, typically 0.5–500 µm in diameter, that have iron oxide particles (or other particles that may be attracted to magnets) contained within the polymer matrix. Bioreactive molecules can be adsorbed or coupled to their surface, and used to separate biological materials such as cells, proteins, or nucleic acids; by the use of magnets or magnetic fields, the beads can be easily manipulated in test tubes or 96-well plates. These microbeads are used for isolation and handling of specific material or molecules, as well as for analyzing sensitive molecules or those that are in low abundance (for example, in miniaturized and automated settings).

Make-up gas: Extra carrier gas or other gas added to the carrier gas as it flows into or through a detector. Make-up gas serves to improve peak shapes for open-tubular columns with detectors not necessarily designed for them exclusively by reducing the effects of detector dead volume. Also, make-up gas may play an active role in detector operation, as for example when hydrogen serves as both make-up and combustion gas in a flame ionization detector.

Mass spectrometric (MS) detector: Chromatography detector that records mass spectra of solutes as they are eluted from the column.

Mass transfer (inter-phase) (*C* term): The process of solute movement between the moving and stationary zones. The *C* term of the van Deemter equation is referred to as the interphase mass transfer term. The faster the process of mass transfer the better the efficiency of column. In HPLC, slow mass transfer is the most important factor affecting column efficiency. Its rate can be increased by the use of small-particle packings, thin layers of stationary phase, low viscosity mobile phases, and high temperatures.

Matrix: In sample preparation, the matrix normally refers to the substance in which the analyst is attempting to measure a solute or series of solutes; often the matrix is of no interest, and its concentration must be reduced or eliminated for a separation and measurement to take place; the matrix can be organic, inorganic, or biological.

Matrix adsorption mode (SPE): A lesser used mode of SPE where the sorbent is chosen to maximize retention of the matrix and other interferences while the analyte of interest is unretained; the opposite of the normal process (bind–elute) of SPE; there is no concentration of the analytes.

Matrix solid phase dispersion (MSPD): Technique uses bonded phase solid supports as abrasives to produce disruption of sample architecture and as a bound solvent to aid complete sample disruption during the sample blending process; the finely divided sample is gently blended with a mortar and pestle, transferred to a column, and the analytes eluted with appropriate solvents.

Matrix-solid phase extraction (MASE): See ***matrix solid phase dispersion.***

Maximum allowable operating temperature (MAOT): Highest continuous column operating temperature that will not damage a column, if the carrier gas is free of oxygen and other contaminants. Slightly higher temperatures may be permissible for short periods of time during column bake-out.

McReynolds constants: System for stationary-phase characterization. McReyonolds expanded on the earlier Rohrschneider polarity probes. The retention indices of a series of test probes such as benzene, 1-butanol, methyl-*n*-propylketone, nitropropane, and pyridine are taken together to express the overall phase polarity, or separately to express the stationary-phase behavior toward individual compound classes.

Mean pore diameter: The average pore diameter of the pore in a porous packing. It is most commonly determined by BET analysis and is reported as four times the specific pore volume divided by the specific surface area based on the assumption of uniform cylindrical pores. The pore diameter is important in that it must allow free diffusion of solute molecules into and out of the pore so that the solute can interact with the stationary phase. Additionally, the pores must be well-connected, with a minimum of dead ends, so that there are many paths that can allow a molecule to access any part of the pore space. In SEC, the packings have different pore diameters and therefore molecules of different sizes can be separated. For a typical substrate such as silica gel, 60- and 100-Å pore diameters are most popular. For packings used for the separation of biomolecules, pore diameters > 300 Å are used.

Megapascal (MPa): A unit of pressure; 1 MPa = 10 bar, 10.133 atm, or 145.0 psi.

Megapores: See ***perfusion chromatography.***

Membrane extraction with sorbent interface (MESI): A version of dynamic headspace where a silicone hollow fiber membrane is placed in the headspace about the sample; an inert gas is passed through the membrane and analytes that are permeable to the membrane pass from the headspace and are swept to an adsorbent trap; after a period of concentration, the trapped analytes are thermally desorbed to the GC column.

Membrane filtration: Membrane filter; membrane disk.

Membrane suppressor: Continuous chemical suppression. Ion exchange through ion-exchange membranes. H^+ or OH^- are supplied by the respective regeneration solution (for example, sulfuric acid).

Metal affinity chromatography: A special form of ligand exchange chromatography used for the separation of biopolymers with a particular affinity for a specific metal cation typically copper(II), zinc(II), and iron(II).

Metalophile: A compound that has high affinity for active (acidic) silanol groups on silicas surface. Usually a strongly basic amine.

Method detection limit (MDL): The minimum amount of solute that can be analyzed within specified statistical limits of precision and accuracy, including sample preparation.

Method development: A process of optimizing the separation including the sample pretreatment so as to obtain a reproducible and robust separation. Usually it emphasizes the search for the stationary phase, eluent, and column temperature combination that provides an adequate separation.

Method translation: Several mathematical techniques for adjusting GC method parameters for variations in the carrier gas type or column dimensions, with the objective of maintaining either the same or ratiometric retention times. Useful when changing from helium to hydrogen carrier gas, or when increasing speed of analysis or resolution by adjusting column dimensions. Not useful if changing the stationary phase to a chemically different type.

Method validation: A process of testing a method to show that it performs to the desired limits of precision and accuracy in retention, resolution, and quantitation of the sample components of interest.

Micellar chromatography: The addition of micelles to the mobile phase to effect separations. The micelles may act as displacing or partitioning agents and provide another parameter which may be used to change selectivity. Surfactants above their critical micelle concentration are used in micellar chromatography and in MEKC form of CE.

Micro LC: Refers collectively to techniques where a column of smaller than conventional internal diameter is used for separation. The term micro LC is most often used for HPLC in <0.5-mm i.d. columns; micro LC is used in high sensitivity analysis when the sample amount is limited and with certain ionization techniques in LC–MS where the volume of solvent flowing into the ionization source must be minimized.

Microbore: Refers to the use of columns with smaller-than-usual internal diameters in HPLC. Columns with internal diameters of 2 mm and below are considered to be microbore columns; columns with internal diameters below 0.5 mm are referred to as micro LC columns. In GC, microbore may signify columns with inner diameters less than 200 μm.

Microchip devices: Microdevices based on silicon, glass, and other types of microfabricated chips where experiments can be miniaturized into single- or multichannel microfluidic circuits; these devices can be used for CE and CEC, and should be low cost and disposable. Microchip-based GC devices have been available since approximately 1995. The use of these devices for separations is

currently in its infancy, and applications should expand with time.

Microdialysis: Microdialysis is a minimally invasive sampling technique that is used for continuous measurement of free, unbound analyte concentrations in the extracellular fluid of virtually any tissue. Analytes may include endogenous molecules (for example, neurotransmitters, hormones, and glucose) to assess their biochemical functions in the body, or exogenous compounds (for example, pharmaceuticals) to determine their distribution within the body. The microdialysis technique requires the insertion of a small microdialysis catheter (also referred to as microdialysis probe) into the tissue of interest. After the probe is inserted into the tissue or (body) fluid of interest, small solutes can cross the semipermeable membrane by passive diffusion. The microdialysis probe is designed to mimic a blood capillary and consists of a shaft with a semipermeable hollow fiber membrane at its tip, which is connected to inlet and outlet tubing.

Microextraction: The general process of liquid extraction using small amounts of organic solvent where the phase ratio V_o/V_{aq} is quite low; other techniques using hollow microfibers as a barrier are also referred to as microextraction.

Microparticulate: Refers to the small particles used in HPLC. Generally packings with a particle diameter of less than 10 µm and that are totally porous are considered microparticle packings.

Micropipette tip: A form of SPE in which the packing material is embedded or adsorbed on the inner walls of a pipette tip; useful for the SPE of very small amounts of liquid sample; often used with *xyz* liquid handling systems for automation purposes. See ***pipette tip.***

Microporous resin: Same as ***microreticular resin.***

Microreticular resin: Cross-linked synthetic ion-exchange resins that have pores with openings corresponding to molecular sizes. Diffusion into the narrow pores can be impaired and low exchange rates can occur, as well as poor performance, especially for large molecules.

Microwave-assisted extraction (MAE): The use of microwave energy to heat samples in the presence of a solvent allowing for rapid extraction; MAE can be performed in open vessels. A nonmicrowave-absorbing solvent is used and the sample containing a substance with a high dielectric constant (for example, water) is rapidly heated, with the extracted analytes passing into the extraction solvent. A variation of this technique involves the addition of an inert microwave-absorbing solid substance that transfers the heated energy to the surrounding solvent. MAE can also take place in closed vessels that are non-microwave-absorbing containers.

Migration time (t_M): The time it takes for a charged molecule to move from the point of injection to the point of detection in a CE capillary.

Milling: Devices for reducing the particle sizes of solid materials. Disk mills pulverize <20-mm diameter hard samples by feeding between stationary and rotating disks with adjustable gap settings; samples are generally reduced to a 100-µm diameter; rotor speed mills combine impact and shearing processes to grind soft-to-medium hard and fibrous materials to 80 µm; ball mills grind material to submicrometer size by developing high-grinding energy via centrifugal or planetary actions using

agate, tungsten carbide, or PTFE-coated stainless steel balls.

Mincing: The process of breaking down a meat or vegetable product into smaller parts by tearing, chopping, cutting, dicing, and so forth.

Minimum detectable quantity (MDQ): The amount of solute that produces a signal twice the noise level.

Mixed-bed column: Combination of two or more stationary phases in the same column, used most often for exchange separations (IEC mixed anion and cation resins) and SEC (mixture of different pore size packings). The advantage in IEC is the total removal of both cationic and anionic compounds; the technique is useful in SEC because a wider molecular weight range can be accommodated by the same column.

Mixed-mode separation: A separation that takes place in a single column as a result of retention and selectivity provided by a dual retention mechanism. For example, at intermediate-to-high pH values, a reversed-phase column with residual silanols can separate by hydrophobic interactions as well as ionic interactions by virtue of the ionized silanols; sometimes mixed-mode separations can be quite beneficial to the selectivity (band spacing) but can cause peak asymmetry; the precise balance of interactions may be difficult to reproduce with subsequent batches of packing.

Mobile phase: The fluid that moves solutes through the column. In LC, the mobile phase interacts with both the solute and the stationary phase and therefore can have a powerful influence on the separation. In GC, the mobile phase, as an inert gas, has little interaction with stationary phase and analytes and serves to move the sample through the column.

Mobile-phase modifier: Modifiers are materials (usually organic or inorganic compounds) added to the mobile phase to alter its elution properties.

Mobile-phase strength: See ***solvent strength.***

Modifier: An additive that changes the character of the mobile phase. In reversed-phase LC, for example, water is the weak solvent and methanol, the strong solvent, is sometimes called the modifier; sometimes other additives such as competing bases like triethylamine or ion pair reagents are referred to as modifiers but they should more correctly be called additives. See ***additive***.

Molecular diffusion term (*B* term): Refers to the *B* term (second term) of the van Deemter and Golay equations. Also called *longitudinal* or *axial diffusion term*. It dominates band broadening only at very low flow rates below the minimum plate height where the diffusion of individual solutes can occur in a longitudinal (lengthwise) direction on the column. See ***van Deemter equation, Golay equation.***

Molecular sieve: GC column packing that retains solute by combined molecular size and adsorptive interactions. Molecular sieves can separate light gases and hydrocarbons.

Molecular weight distribution: The distribution of the molecular weight of molecules in a polymer sample. Distribution can be defined as weight average and number average.

Molecularly imprinted phases (MIPs): See ***imprinted phases.***

Monolith: A monolithic HPLC column is a special type of column used in HPLC with porous channels rather than beads; monoliths, in chromatographic terms, are porous rod structures characterized by mesopores

and macropores. These pores provide monoliths with high permeability, a large number of channels, and a high surface area available for reactivity. The backbone of a monolithic column is composed of either an organic (polymeric) or inorganic (silica) substrate, and the column can be chemically altered for specific applications.

Monomeric phase: Refers to a bonded phase where single molecules are bonded to a support. For silica gel, monomeric phases are prepared by the reaction of an alkyl- or arylmonochloro- or alkoxysilane. Polymeric phases generally are prepared from a di- or trichlorosilane or an alkoxysilane reactant.

Moving zone: The moving zone is that fraction of the mobile phase in the column that occupies the interstitial spaces.

Multidimensional chromatography: The use of two or more columns or chromatographic techniques to effect a better separation. It is useful for sample cleanup, increased resolution, and increased throughput. Separation is carried out with two or more columns in which peaks are selectively directed onto or removed from at least one of the columns by use of a timed valve system. In GC, a Deans fluidic switch is often used. It also can be used off-line by collecting fractions and reinjecting onto a second column. Also called *coupled column chromatography, column switching, multicolumn chromatography,* and *boxcar chromatography*. See ***backflushing, heart cutting, precut.***

Multidimensional protein identification technology (MudPIT): Multidimensional protein identification technology combines both a cation-exchange prefractionation and reversed-phase HPLC separation of tryptic peptides to analyze an entire proteome of a cell or tissue type protein extract. The approach uses a dual enzymatic digestion (Lys-C followed by trypsin) to increase the number of peptides observed. The peptides are separated using strong cation exchange and are identified by MS-MS detection.

Multimodal SPE: The practice of SPE where two different phases or modes are used to clean up a sample; the process can consist of two separate cartridges placed in series with the analytes separated on the two different cartridges; a second process is where two different phases are present in the same cartridge or even on the same packing; sometimes referred to as *mixed-mode SPE*.

N

Nano LC: LC practiced with columns that have internal diameters less than 100 µm; usually requires specialized instrumentation; often used in proteomic studies where sample is limited and sensitivity is required.

Narrow-bore column: Columns of less than 2 mm i.d. used in HPLC, and less than 320 µm i.d. in GC; also referred to as ***microbore***.

Nitrogen–phosphorus detection (NPD): The nitrogen–phosphorus detector catalytically ionizes N- or P-containing solutes on a heated rubidium or cesium surface in a reductive atmosphere. NPD is highly selective with sensitivity somewhat better than FID.

Noise: See ***baseline noise.***

Non-aqueous reversed phase chromatography (NARP): Refers to reversed-phase chromatography performed without water as a component of the eluent on a reversed-phase packing. Used for compounds that are very nonpolar that

either cannot be eluted or are poorly eluted from a reversed-phase column with 100% methanol or acetonitrile; in these cases, solvent A would be acetonitrile and solvent B would be a stronger solvent such as tetrahydrofuran; for NARP, reversed-phase rules apply (that is, the more nonpolar the analyte, the greater the retention).

Nonpolar: A nonpolar molecule is one in which the electrons are distributed more symmetrically and thus does not have an abundance of charges at the opposite sides. The charges all cancel out each other. Nonpolar compounds, solvents, or bonded phases readily dissolve in organic solvents, such as hexane, or prefer such solvents in place of water. Nonpolar substances do not readily dissolve in water.

Nonporous particle: Refers to a solid particle used as a support for a porous coated or bonded phase; pellicular particles are nonporous particles with large particle diameters (approximately 40 µm) and nonporous silicas and resins with small particle diameters of less than 3 µm usually consist of a microbead with a thin porous outer coating of silica gel, bonded silica gel, or polymeric phase.

Nonsuppressed ion chromatography: Direct ion chromatography. After the separation column the eluent is directly fed into the conductivity detection without prior chemical suppression. The conductivity measurement takes place on the high background conductivity. This requires a very high quality detector with perfectly stable temperature. The nonsuppressed approach allows detection of weak acids or bases that are undissociated after suppression — for example, silicate or borate. For cation analysis the peaks for the components of interest are larger than with suppression.

Nonporous packing (NPR, NPS, NPZ): Particles similar to porous-layer bead but with particle diameters in the sub-5-µm range, often particles are in the sub-2-µm range; used for high-speed separations in short columns; NPS refers to nonporous silica, NPR to nonporous resins, and NPZ to nonporous zirconia.

Normal-phase chromatography: A mode of chromatography in which the stationary phase is more polar than the mobile phase. Adsorption chromatography on silica gel or alumina using mixtures of less polar eluents (for example, hexane–diethethyl ether) as a mobile phase would be a typical normal-phase system. Also refers to the use of polar bonded phases, such as -CN or NH_2. Sometimes referred to as *straight phase chromatography*.

O

Octadecylsilane (ODS, C18): The most popular reversed phase in HPLC. Octadecylsilane phases are bonded to silica or polymeric packings. Both monomeric and polymeric phases are available.

Octylsilane (C8): A popular stationary phase in reversed-phase chromatography; usually has slightly less retention than the more popular C18; both monomeric and polymeric phases available

Off-line SPE: The normal practice of SPE where SPE cartridges, disks, pipette tips, and so forth are handled using manual processes (for example, vacuum manifolds or pipette transfer); opposite to on-line SPE.

On-column detection: The column itself serves as the flow cell in HPLC, CE, or CEC. Generally the term used when fused-silica capillaries are employed; the outer polyimide layer is removed and optical beam is directed through the capillary; a measuring device (for example, a

photomultiplier tube) is located on the opposite side of the capillary.

On-column injection (OCI): In GC, refers to the process of injecting the entire liquid sample directly onto the head of the column using a fine needle that will fit inside the capillary. Usually carried out at an initial column temperature less than the solvent boiling point, also termed *cold on-column injection*.

On-line column switching: See ***multidimensional chromatography, on-line SPE.***

On-line preconcentration: A precolumn is placed in front of the separation column to concentrate analytes before their separation; different mechanisms may be used (for example, hydrophobic interaction, adsorption, or enzymatic reaction) to retain analytes as a function of time and then by a displacement process (such as solvent elution or pH change) concentrated analytes are transferred to the separation column.

On-line SPE: Refers to the use of small stainless steel cartridges packed with SPE packings placed across two ports of a 6- or 10-port injection or column-switching valve. The SPE trap is loaded with sample by an external pump or syringe transfer and then the valve is switched so that the SPE trap becomes part of the HPLC flow stream and analytes can be swept into the column based on the solvent being used for displacement. On-line SPE columns are usually used multiple times whereas off-line SPE cartridges are generally used once.

Open-tubular column: Also termed capillary columns, open-tubular columns for GC have the stationary phase coated or chemically bonded on the inner walls or have support particles deposited on the inner walls. Internal diameters range from ~100 µm up to 750 µm. In HPLC, SFC, and capillary electrophoresis, small internal diameter (less than 100 µm) columns are used. The most frequently used column material is fused-silica tubing. Used very little in routine HPLC or SFC but routinely in CE and GC. Also termed ***capillary column.***

Open-tubular column, packed: A capillary-dimension column that is packed with stationary phase particles. Also called *micropacked columns*, particularly in GC.

Optically active resin: Incorporation of optically active groups into an ion-exchange resin to allow separation of optically active isomers. Not many are commercially available in HPLC.

Organic modifier: Water-miscible organic solvent added to an aqueous mobile phase to effect separations in reversed-phase HPLC. Common organic modifiers are acetonitrile, methanol, isopropanol, and tetrahydrofuran.

Orthogonality: Refers to two separation dimensions for which the elution times in the two dimensions can be treated as statistically independent; ideally, the two dimensions should have totally different retention mechanisms (for example, reversed phase and normal phase; ion exchange and reversed phase; polar and nonpolar)

Overload: In preparative chromatography, the overload is defined as the sample mass injected onto the column where efficiency and resolution begins to be affected if the sample size is further increased. See ***sample capacity.***

P

Packing: The adsorbent, gel, or solid support used in the chromatography column. Most modern analytical HPLC packings are less than 10 µm in average diameter

with 5 µm currently the favorite. GC column packings range in size from 60–80 mesh down to 100–120 mesh.

Paired ion chromatography: The same as ***ion-pair chromatography.***

Paper filtration: Using porous filter paper (mainly cellulose) to remove particulates from liquid samples; papers with different porosities are available. Low porosity filters will remove very fine particulates but may have a lower flow rate while high porosity filters filter out larger particulates at a higher flow rate; paper filtration is often used in wet chemistry to filter, combust, and then weigh insoluble materials; ashless filter paper is used for this purpose.

Particle diameter (d_p): Average diameter of the column packing particles.

Particle size distribution: A measure of the distribution of the particles used to pack the LC column. In HPLC, a narrow particle size distribution is desirable. A particle size distribution of d_p ± 10% would mean that 90% of the particles fall between 9 and 11 µm for a 10-µm average d_p packing.

Particle size reduction: The general process of reducing larger particles down to a size that can be more conveniently extracted; the smaller the particle the more quickly it will dissolve or if insoluble the more quickly analytes can be extracted for further sample cleanup. Typical methods for reducing particle size include pulverizing, milling, homogenizing, chopping, blending, and so on.

Particulates: Generally refers to a small particles found in the mobile phase that can cause back pressure problems by lodging in frits; it can also refer to the small particles packed into HPLC columns. Particulates that escape from the column exit may cause detector noise.

Partition chromatography: Separation process where one of two phases is held stationary on a solid support or the column inner wall (stationary phase or liquid phase) while the other is allowed to flow freely down the column (mobile phase or carrier gas). Solutes partition themselves between the two phases based on their individual partition coefficients. LLC is an example; modern bonded-phase LC can be considered to be a form of partition chromatography where one of the liquid phases is actually bonded to the solid support. Mechanistically, partition chromatography implies that the solute becomes at least partially embedded within the stationary phase, which is impregnated, coated, or bonded to the substrate, in contrast to an adsorption process in which the solute does not penetrate into the retentive surface or interphase.

Partition coefficient (K): The ratio of the equilibrium concentration of solute in the stationary phase relative to the equilibrium concentration of solute in the mobile phase. In GC, the relative concentration of solute in the mobile and stationary phases is a function of k and β: $K = \beta k$. Also called *distribution coefficient*, K_D, and *distribution constant*, K_c.

Passive sampling: In passive gas sampling, an air sample is pulled through a flow controller into an evacuated canister over a chosen period of time, ranging from 5 min to 24 h. The sampling period and the flow rate determine the canister volume required.

Peak: The profile of an analyte compound as it is eluted from a column through a detector; usually depicted on a visual output on a recorder or printer

based on the detector's electrical response.

Peak area (A_p): The area measured under a chromatographic peak; usually measured by an integrator or data system; the peak area is related to the amount of substance eluted in a peak.

Peak capacity (n): The number of equally well resolved peaks that can be fit in a chromatogram between the hold-up volume and some upper limit in retention k_n. For $R = 1$, n can be expressed by the approximation $n = 1 + \sqrt{N}/4 \cdot \ln(1 + k_n)$ where N is the plate number and k_n is the retention factor of peak n.

Peak dispersion: See ***band broadening***.

Peak doublet: A split peak generally caused by column void, poor injection technique, or solvent flooding in GC. Split peaks also could be closely eluted compounds.

Peak height (h_p): The maximum height of a chromatographic peak as measured from the baseline to the peak apex; the peak height is related to the amount of substance eluted in a peak.

Peak overload: When too much of any one solute is injected its peak may be distorted into a triangular shape.

Peak shape: Describes the profile of a chromatography peak. Theory assumes a Gaussian peak shape (perfectly symmetrical); peak asymmetry factor describes shape as a ratio. See ***asymmetry.***

Peak tracking: A method of matching of peaks that contain the same compound between different experimental runs during method development; relies upon detection parameters of each pure analyte; diode-array detectors and mass spectrometers are among the best detectors for peak tracking because of their specificity. Also refers to data-system tracking of gradual changes in retention times caused by stationary-phase loss or other column degradation or drift in the chromatographic conditions.

Peak variance (σ^2): The second central moment of the peak about the retention time. For a Gaussian peak the variance is the fundamental parameter controlling peak width. See ***Gaussian peak.***

Peak volume (V_p): The volume occupied by a chromatographic peak from starting basepoint to ending basepoint as it passes through the detector: $V_p = F_c w_b$

Peak width at base (w_b): The width of the chromatographic peak at the baseline as eluted from the column. It is measured at the baseline by drawing tangents to the inflection points on the sides of the Gaussian curve representing the peak. Smaller peak widths usually represent efficient separations; also referred to as *band width*. It is sometimes convenient to estimate the peak width at base from the peak area and height: $w_b = 1.596\, A_p/h_p$ (see Figure 2).

Peak width at half-height (w_h): The width of the chromatographic peak at half of the peak height above the baseline. Smaller peak widths usually represent efficient separations; also referred to as *band width*. It is sometimes convenient to estimate the peak width at half-height from the peak area and height: $w_b = 0.94\, A_p/h_p$ (see Figure 2).

PEEK: Polyether ether ketone (PEEK) is a colorless organic polymer. It is used as a material for inert capillaries and fittings in HPLC and IC systems

Pellicular: See ***porous layer bead.***

Percent B (%B): Refers to the stronger solvent in a binary solvent mixture; %A would be the weaker solvent analog.

Perfusion chromatography: Refers to chromatography performed using particles with very large pores (for example, 4000–8000 Å)

called throughpores (megapores or gigapores). Eluent flows through the particle as well as smaller interconnecting pores (for example, 300–1000 Å) called diffusive pores between the large pores. Best suited for the preparative separation of macromolecules.

Permeability (B_o): Also referred to as *column permeability* and *specific permeability*; a term expressing the resistance of the packed column to the flow of mobile phase. For a packed column: $B_o = (d_p^2/180) \cdot \varepsilon^3/(1 - \varepsilon)^2 \approx (d_p^2)/1012$.For an open-tubular column: $B_o = d_c^2/32$. A column with high permeability gives a low pressure drop.

Permeation: In SEC, refers to the process where a solute can enter a mobile phase filled pore of the packing.

Phase collapse: See ***phase dewetting.***

Phase dewetting: A term used in reversed-phase LC where very dense bonded-phase coverage and a high percentage of aqueous content in mobile phase can lead to expulsion of water from the pores, which prevents the normal partitioning process from taking place. Phase dewetting may occur with as high as 10% organic content in the mobile phase but can occur at lower %B values; results in earlier than normal elution of analytes, poor peak shape, and nonreproducible retention times.

Phase ratio (β): The relative amount of stationary to mobile phase in the column. In partition chromatography: $\beta = V_S/V_M$ where V_S and V_M are the volume of stationary and mobile phase in the column respectively. For open-tubular columns: $\beta \approx r_c/2d_f$. Thicker stationary phase films or higher phase loading gives longer retention and higher peak capacity.

Phenol extraction: A sample preparation technique used for the isolation of DNA from biological samples.

Phenyl phase: A popular nonpolar bonded phase prepared by the reaction of dimethylphenylchlorosilane or alkoxysilane with silica gel for LC, or as the components of a cross-linked or bonded phase for GC. Claimed to have affinity for aromatic-containing compounds and does impart a different selectivity compared to alkyl bonded phases.

Photoionization detection (PID): The photoionization detector ionizes solute molecules with photons in the UV energy range. PID is a selective detection method that responds to aromatics and olefins when operated in the 10.2-eV photon range. It can respond to other materials with a more energetic light source.

PIONA: Refers to the analysis of paraffins, isoparaffins, olefins, naphthenes, and aromatics.

Pipette tip: Replaceable tips used in automation of liquid handling chores; used once and discarded to avoid contamination.

Pirkle column: Chiral "brush type" stationary phases based on 3,5-dinitrobenzoyl-phenylglycine silica that are used in the separation of a wide variety of enantiomers. Named after the developer, Dr. William Pirkle, University of Illnois.

pK_a: An acid dissociation constant, K_a, (also known as acidity constant, or acid-ionization constant) is a quantitative measure of the strength of an acid in solution. It is the equilibrium constant for a chemical reaction known as dissociation in the context of acid-base reactions. The equilibrium can be written symbolically as: $HA \leftrightarrow H^+ + A^-$ where HA is a generic acid that

dissociates by splitting into A^-, known as the conjugate base of the acid, and the hydrogen ion or proton, H^+, which, in the case of aqueous solutions, exists as the hydronium ion — in other words, a solvated proton. The dissociation constant is usually written as a quotient of the equilibrium concentrations (in mol/L), denoted by [HA], [A^-], and [H^+]: $K_a = ([H^+] [A^-])/[HA]$; due to the many orders of magnitude spanned by K_a values, a logarithmic measure of the acid dissociation constant is more commonly used in practice. The logarithmic constant, pK_a, which is equal to $-\log_{10} K_a$, is sometimes also (but incorrectly) referred to as an acid dissociation constant.

Planar chromatography: A separation technique in which the stationary phase is present as or on a plane (IUPAC). Typical forms are paper and thin layer chromatography.

Plate height (*H*): See ***theoretical plate height.***

Plate height, effective (Heff): The column length divided by the number of effective theoretical plates: $H_{eff} = L/N_{eff}$

Plate number (*N*): See ***theoretical plate number.***

Polar: A polar molecule may be polar as a result of polar bonds or as a result of an asymmetric arrangement of nonpolar bonds and nonbonding pairs of electrons; polar molecules are generally able to dissolve in water (H_2O) because of the polar nature of water; polar molecules do not prefer nonpolar organic solvents such as hexane. Polar molecules have slightly positive and slightly negatively charged ends; we often refer to a compound's polarity.

Polarity index (*P′*): The polarity index is a measure of the relative polarity of a solvent and is useful for identifying suitable mobile phase solvents or extraction solvents. The polarity index increases with polarity; examples: hexane, $P' = 0.0$; isopropanol, $P' = 3.9$; tetrahydrofuran, $P' = 4.0$; methanol, $P' = 5.1$; acetonitrile, $P' = 5.8$; water, $P' = 9.0$

Polyacrylamide gel: Neutral hydrophilic polymeric packings used in aqueous SEC. They are prepared by the copolymerization of acrylamide with *N*,*N′*-methylene-bis-acrylamide.

Polyaromatic hydrocarbon (PAH): Members of a class of hydrocarbon molecules characterized by one or more fused aromatic rings.

Polychlorinated biphenyl (PCB): Biphenyl molecule with two or more chlorine substitutions.

Polyethylene glycol (PEG): Polymeric hydrocarbon used as a GC stationary phase; possesses moderately polar retention characteristics.

Polyethyleneimine (PEI): Polyethyleneimine, an anionic polymeric phase used to coat or bond onto silica or a polymeric packing. Most often used for the separation of proteins and peptides.

Polymeric packings: Packings based on polymeric materials, usually in the form of spherical beads. Typical polymers used in LC as well as GC are polystyrene–divinylbenzene (PS-DVB), polydivinybenzene, polyacrylamide, polymethylacrylate, polyethyleneoxide, polydextran, or polysaccharide.

Polymeric phase: Refers to chemically bonded phase where a polymer species is bonded to silica-based particles or to the wall of an open-tubular column.

Polymeric SPE: The use of a polymeric base material (for example, PS-DVB or methacrylate) rather than an inorganic material (for example, silica or alumina); polymers generally have a wider

pH range and higher sample capacity than some of the inorganic materials.

Polystyrene–divinylbenzene (PS-DVB) resin: The most common base polymer for ion-exchange chromatography. Ionic groups are incorporated by various chemical reactions. Neutral PS-DVB beads are used in reversed-phase LC. Porosity and mechanical stability can be altered by variation of the crosslinking through the variation of the DVB content. In GC, a number of porous polymer stationary phases are available for gas and light-compound separations.

PONA: Refers to the analysis of paraffins, olefins, naphthenes, and aromatics.

Poppe plot: A kinetic plot named after Professor Hans Poppe (*J. Chromatogr. A* **778,** 3 [1997]), University of Amsterdam, the Netherlands, where the plate time log (t_0/N) is depicted as a function of the number of theoretical plates to assess the limits of column performances as a function of particle size, column pressure drop, and so forth.

Pore diameter: Same as ***mean pore diameter.***

Pore size: The average size of a pore in a porous packing. Its value is expressed in angstroms or nanometers. The pore size determines whether a molecule can diffuse into and out of the packing. See ***mean pore diameter.***

Pore volume (V_i): The total volume of the pores in a porous packing, usually expressed in mL/g. Better termed the specific pore volume. It is measured by the BET method of nitrogen adsorption or by mercury intrusion where Hg is pumped into the pores under high pressure.

Poroshell: Similar to nonporous particles and porous-layer beads; particles are generally in the 2–5 µm range with a submicrometer-thick shell; wide pore versions (>300 Å) allow rapid diffusion of macromolecules and smaller pore versions (90–120 Å) are for small molecules; give similar efficiency to sub-2-µm particles but at much lower pressure because of their larger particle size.

Porosity: For a porous substrate, the ratio of the volume of the pores in a particle to volume occupied by the particle. The pore volume is a measure of the porosity and is expressed in mL/g.

Porous layer bead: A small glass bead coated with a thin layer of stationary phase. The thin layer can be an adsorbent, resin, or a phase chemically bonded onto the adsorbent. These packings were among the first to be used in HPLC. They were of larger particle size (20–40 µm) than the microparticulate packings of today but were easy to pack and gave adequate efficiency. Also referred to as *controlled surface porosity supports* and *pellicular materials*.

Porous particle: Refers to column packing particles possessing interconnecting pores of specified diameter and pore volume; generally in HPLC porous particles with diameters below 10 µm are the most popular, and in preparative chromatography larger particles are used because of their lower cost and higher column permeability.

Porous polymer: A packing material, generally spherical, based on organic polymers or copolymers; popular examples would be polystyrene–divinylbenzene, polyacrylates, polydextrans, polyacrylamides, and polybutadienes. Retains solutes by selective adsorption or molecular size interaction.

Porous-layer open-tubular (PLOT) column: An open-tubular column used in GC or HPLC that has particles coated or uncoated with stationary phase

attached to the inner walls, which allows more rapid mass transfer. In GC, small porous particles such as polymer, alumina, silica, and so forth are attached to the walls or the wall may be modified by etching or other treatment to increase the inner surface area and provide gas–solid chromatographic retention behavior. In LC, porous polymers have been used occasionally. In GC, the technology is more developed.

Postcolumn derivatization: See ***postcolumn reaction.***

Postcolumn reaction: In LC and IC, after the analytical column a UV-transparent ion or molecule is converted into a component with better detectability (that is, UV–vis absorbance, fluorescence) by adding a specific reagent. This product is then detected with UV–vis or fluorescence detection. The reaction of the analyte and the reagent is usually very selective and yields often in a colored product (visible detection), that is, chromate + diphenylcarbazide complex (540 nm); bromate + iodide → triiodide (352 nm). Parameters that will influence the sensitivity are the reaction time (flow rate/length of reaction coil), the reaction temperature, pH, concentration of catalysts. In GC, postcolumn methanization may be used to convert CO and CO_2 to CH_4 with hydrogen and a heated nickel catalyst to achieve flame ionization detection, more sensitive than thermal conductivity detection.

Potentiometric detection: Ion selective electrodes in a flow-through cell are used to detect the analyte ions. Not a very common type of detection.

Pounds per square inch (psi): A unit of pressure: 14.6959 psi = 1 atm = 101.325 kPa = 1.013 bar.

Precolumn derivatization: See ***precolumn reaction.***

Precolumn reaction: Analytes are converted into components with better detectability (for example, UV–vis absorbance) by a chemical reaction before injection. The analytes are separated and detected by UV–vis detection. Complexing agents such as EDTA, NTA, and so forth are used as their Fe(III) complexes.

Precolumn: A short section of similar column placed before the analytical column; used to physically retain undesired compounds or to saturate the mobile phase with stationary phase that may be packed into the precolumn (for example, a silica precolumn saturates the mobile phase with dissolved silica and prevents mobile phase from dissolving silica in the analytical column).

Precolumn filter: A filter used between the injector and the column (or guard column) to keep unwanted sample components from reaching the column; sometimes called *in-line filter*, occasionally *inlet filter*.

Preconcentration: See also ***trace enrichment.***

Precut: Peaks in the beginning of a chromatogram are removed to vent or are directed onto another column of different polarity, or at a different temperature, for improved resolution. See ***heart cutting, multidimensional chromatography.***

Prefilter (SPE): In cases where samples contain a large amount of particulates, regular SPE cartridges and disks may become clogged and flow is reduced. Prefilters are filter devices that have higher porosity that will filter out large particles and allow the SPE bed to operate more efficiently. Some SPE devices have prefilters built in; in others one can add a prefilter. In some cases, the use of an inert packing such as glass beads serves the same purpose as an actual filter.

Preparative chromatography: Refers to the process of using chromatography as a technique for the isolation of a sufficient amount of material for other experimental or functional purposes. For pharmaceutical or biotechnological purifications, large columns of several feet in diameter can be used for purifying multiple grams of material. For isolating a few micrograms of valuable natural product, a 4.6-mm i.d. analytical column can be used. Both of these separations can be considered preparative chromatographic approaches. Preparative LC is often employed; preparative GC is seldom used.

Pressing: The general process of squeezing liquid from a semisolid material (such as plants, fruit, or meat).

Pressure drop (Δ*p*): The pressure drop across a column: $\Delta p = p_i - p_o$, where p_i and p_o are pressure at the column inlet and outlet, respectively.

Pressure injection (CE): Pressure-induced injection; the use of pressure or vacuum to inject small (nanoliter) volumes of sample into a capillary column; best for narrow-bore capillaries (<10 μm i.d.); a version of hydrostatic injection.

Pressure, absolute inlet (p_i): The column inlet pressure expressed relative to a vacuum.

Pressure, absolute outlet (p_o): Pressure at the column outlet, relative to vacuum.

Pressure, back: Same as head pressure, column pressure.

Pressure, head (Δ*p*): The pressure difference between the inlet and outlet of the column. In LC, governed by the following approximate equation for a column packed with spherical particles: $\Delta p \approx (3000 L\eta)/(t_0 d_p^2)$ where η is the mobile phase viscosity, t_0 the column holdup time, and d_p is the particle diameter. In packed-column GC the pressure drop can be approximated as $\Delta p \approx (1012 L\ \eta\ \overline{u})/(d_p^2)$, where $\overline{u}$ is the average linear velocity. In open-tubular column GC, the pressure drop is $\Delta p \approx (8\ L\ \eta\ \overline{u})/(r_c^2)$. The equations for GC will overestimate the required pressure drops by more than 10% at inlet pressures above 400 kPa (58 psig) because of gas compressibility effects. Pressure drop can be expressed in pressure units of psig, bar, atm, kPa, or MPa. The above equations will yield pressures in pascals if the dimensions are expressed in centimeters, times in seconds, and viscosities in pascal-seconds.

Pressure, relative (*P*): Relative pressure across the column: $P = p_i/p_o$

Pressurized-fluid extraction (PFE): Pressured fluid extraction is a liquid–solid extraction process where the sample and solvent are placed in a closed container and heated well above the solvent's normal boiling point. The combination of increased temperature and resultant pressure extracts analytes and matrix compounds into the superheated fluid, often in a few minutes. Because the technique extracts a wide variety of soluble compounds, additional cleanup steps may be required after PFE is completed; method development involves selecting the best solvent for analytes and the poorest solvent for the matrix and other interferences that may be present. The technique has been approved for various environmental samples by the U.S. EPA under the generic name of PFE or ***pressurized-solvent extraction.*** See ***accelerated solvent extraction.***

Pressurized-solvent extraction (PSE): See ***accelerated solvent extraction, pressurized-fluid extraction.***

Primary sampling: The collection of one or more increments or units initially taken from a population; the primary sample is that taken from the

primary source; proper statistical sampling protocols are recommended.

Process-scale chromatography: Refers to the use of liquid chromatography at the industrial scale level outside the laboratory; generally requires specially designed columns (usually with diameters > 5 cm), recoverable solvents, lower-cost packings (with larger and irregular-shaped particles), and overloaded operating conditions compared to those of laboratory-scale HPLC.

Programmed elution: A procedure in which the conditions of separation are changed in a programmed manner. Unlike LC, in GC and SFC both temperature and pressure can be programmed, separately or simultaneously.

Programmed temperature chromatography: Use of conditions in which the temperature is varied during the run in a controlled manner. Widely used in GC; seldom seen in LC.

Programmed temperature injection (PTI): A cold injection technique in which the inlet temperature is specifically programmed from the GC.

Programmed temperature rate: The rate, in °C/min, at which the GC oven temperature is increased during a controlled temperature program ramp. The program may consist of multiple ramps with variable hold times before and after each. Typical GC programming rates range from <0.5 °C/min up to 40 °C/min. Programming rates up to 200 °C/min and higher have been applied to high-speed gas chromatography.

Programmed temperature vaporization (PTV): In PTV, the sample is introduced into the inlet liner at a temperature slightly below the boiling point of the solvent; the solvent is continually evaporated and vented through the inlet split line; after the solvent is gone, the temperature of the inlet is heated very rapidly to transfer the sample into the column; using PTV there is the potential for less sample discrimination and less thermal degradation of sensitive compounds compared to hot inlet injections.

Programmed temperature vaporizer (PTV): An inlet system designed to perform programmed-temperature injection.

Protein crashing: The term used in removing or reducing the protein concentration in a biological fluid such as plasma. After slight dilution, an organic solvent such as acetonitrile is added to the plasma and the proteins, which are insoluble, precipitate (crash). Centrifugation or filtration is used to remove the protein, and the supernatant liquid is injected into an HPLC system or worked up further.

Protein precipitation: See ***protein crashing.***

Pulsating flow: Flow originating from a reciprocating pump. Normally the pulses are dampened out by a pulse damper, an electronic pressure feedback circuit, or an active damper pump head. Some detectors (for example, electrochemical, refractive index) are greatly affected by flow pulsations.

Pulsed amperometric detection: Electrochemical detection applying different potentials (pulses) to the working electrode. Components that can be analyzed include those that are oxidized or reduced at the electrode and those that react with the electrode surface or cover it. To remove reaction products that could foul the electrode, highly oxidative and reductive potentials are applied to the working electrode after the measuring potential. This removes the reaction products from the previous measuring cycle and renews the

electrode surface. Typical applications are carbohydrates and amino acids.

Pulsed discharge detection (PDD): Several ionization detectors use a pulsed-discharge ion source to improve detectivity compared to constant-discharge detectors.

Pulsed-splitless injection: A form of GC injection recommended for large volumes (up to 5 µL) of sample where a short-term high pressure pulse is imposed on the inlet such that there is not a large volume of solvent vapor generated and most or all of the sample is directed to the column; after the sample is transferred, then normal pressure is resumed. Using this technique, highly volatile compounds are less likely to be lost through the split vent line and thermally unstable compounds spend less time in the hot injection port so there is less degradation.

Pulverizing: Electromechanically driven rod or vibrating base devised to reduce the particle size of solid samples. A freezer mill can be used with liquid nitrogen to treat malleable polymers or those with low glass transition temperatures.

Purge-and-trap sampling: Dynamic headspace technique where the headspace vapors over a liquid or solid sample are continuously removed by a flow of gas over the sample (purging) or through the sample (sparging); volatilized analytes are usually concentrated by trapping on an adsorbent or by cryogenic means. The trap is then heated to desorb trapped components into a GC column. Most often used for volatile trace analytes where concentration is needed.

Pyrolysis gas chromatography: The process of heating a sample enough to break its chemical bonds, thereby forming smaller molecules that can be analyzed by GC. Often applied to polymer characterization.

Q

Quaternary methyl amine (QMA): A strong anion-exchange functionality popular in resin-based packings; usually supplied in chloride form.

Quaternary mobile phase: See ***quaternary-solvent mobile phase.***

Quaternary-solvent mobile phase: A mobile phase consisting of four separate solvents that allow the mobile-phase composition to be fine-tuned; most often this mobile phase is delivered by a low-pressure quaternary pump.

QuEChERS: A technique initially used for the extraction of pesticides from fruits and vegetables. It consists of two steps: salting out extraction using buffered or unbuffered solvent (usually acetonitrile), and dispersive SPE where a solid adsorbent is used to treat an aliquot from the first step to remove interferences and matrix compounds. QuEChERS (which stands for quick, easy, cheap, effective, rugged, and safe) is mostly used with GC–MS and LC–MS (or MS-MS) to more selectively analyze pesticide extracts. More recently, QuEChERS has expanded to matrices such as cooking oil, meat, fish, and biological fluids, and to other analytes, such as pharmaceuticals, antioxidants, and toxins.

R

Radial compression: The use of radial pressure applied to a flexible wall column to cut down on wall effects.

Radial diffusion or dispersion: Diffusion or dispersion across the column in a radial direction. If the sample is injected into the exact center of a column, it will spread not only in a longitudinal

direction as it moves down the column but radially as well, allowing the solute to reach the wall region where the eluent velocity is different than in the center of the column.

Recovery: The amount of solute (sample) that is eluted from a column relative to the amount injected. Excellent recovery is important for good quantitation, for preparative separations, especially for biomolecules, and for good peak shape and resolution. Reasons for inadequate recovery can be solute interaction with active sites on the packing, with column frits, and with column tubing. Compound decomposition during the separation process can also effect recovery.

Recycling chromatography: A technique where the column effluent is recirculated onto the head of the column in an attempt to gain the advantage of extended column length. Can be carried out on a single column by passing the effluent back through the pump. An alternative technique uses two columns connected by a switching valve where the effluent of one column is directed onto the head of the other column. Very seldom used in HPLC, and then only in exclusion chromatography.

Reduced plate height (*h*): The plate height expressed in terms of the average particle diameter for packed columns: $h = H/d_p$ where d_p is the particle diameter, or in terms of the column inner diameter for open-tubular columns: $h = H/d_c$ where d_c is the column inner diameter.

Refractive index detection (RI detection): Based on differential refractive index between the mobile-phase solvent and the eluted analyte in mobile-phase background; not useful in gradient work; often used in size-exclusion chromatography.

Refractive index peak: A pseudo peak normally found near the hold-up volume resulting from the refractive index sensitivity of absorbance and other detectors.

Regeneration: Regeneration of the packing in the column to its initial state after a gradient elution. Mobile phase is passed through the column stepwise or in a gradient. The stationary phase is restored (solvated) to its initial condition. In ion exchange, regeneration involves replacing ions taken up in the exchange process with the original ions which occupied the exchange sites. Regeneration can also refer to bringing back any column to its original state (for example, the removal of impurities with a strong solvent).

Relative retention (*r*): Retention relative to a standard peak: $r = t'_R/t'_{R(st)}$ where t'_R is the retention time of the component of interest and $t'_{R(st)}$ is the retention time of the standard peak. Also: $r = k_i/k_{st}$ where k_i and k_{st} are the corresponding retention factors. For two adjacent peaks, the separation factor, α, expresses the relative retention. See ***separation factor, resolution***.

Relative standard deviation (RSD, %RSD): In probability theory and statistics, the relative standard deviation (RSD or %RSD) is the absolute value of the coefficient of variation. It is often expressed as a percentage. A similar term that is sometimes used is the *relative variance* which is the square of the coefficient of variation. Also, the relative standard error is a measure of a statistical estimate's reliability obtained by dividing the standard error by the estimate; then multiplied by 100 to be expressed as a percentage. The relative standard deviation is widely used in analytical chemistry to express the precision and repeatability of an assay.

Removable well plates: See ***array 96-well plate.***

Representative sample: A sample resulting from a statistically worked out sampling plan; it can be expected to adequately reflect the properties of interest of the parent population.

Residual silanols: The silanol (-Si–OH) groups that remain on the surface of a packing after chemically bonding a phase onto its surface. These silanol groups, that may be present in very small pores, may not be accessible to the reacting bulky organosilane (such as octadecyldimethylchlorosilane) but may be accessible to small polar compounds. Often they are removed by endcapping with a small organosilane such as trimethylchlorosilane. See ***endcapping***.

Resin: A solid polymeric packing used in ion exchange separations. The most popular resins are polystyrene–divinylbenzene copolymers of small particle size (<10 µm). Ionic functionality is incorporated into the resin.

Resolution (R_s): Peak resolution; incorporates both efficiency and separation. A resolution of 1.5 is said to be "baseline" resolution, and a minimum resolution of 1.7–2.0 is considered essential for robustness. For two closely eluted peaks: $R = (t_{R,2} - t_{R,1})/w_b$ where the subscripts 1 and 2 refer to the first and second peaks. From N, k_2 and α: $R_s = (\sqrt{N}/4)((\alpha - 1)/\alpha)(k_2/(k_2 + 1))$ (k_2 is the retention factor of the second peak).

Resolution equation: See ***resolution.***

Response factor (RF): Defines the relationship between the measured peak area or height and the quantity of substance represented by a peak.

Restricted access media (RAM): RAM are sorbents are used for the direct injection of biological fluids such as plasma or serum into an HPLC flow stream. They contain an outer hydrophilic surface that provides minimal interaction with proteins and when combined with small pores on the sorbent exclude the proteins. The inner surface is hydrophobic, and when small molecules diffuse into the pores they interact by reversed-phase mechanisms and are retained. The small molecules such as drugs and their metabolites can be removed by rinsing with an organic solvent. RAMs are most successfully used in a column switching setup where the secondary column is used to resolve the small molecules and the proteins are directed to waste so as not to foul the secondary column.

Retention factor (k): The measure of time the sample component resides in the stationary phase relative to the time it resides in the mobile phase: $k = (t_R - t_M)/t_M$. Formerly, k' was used and it was called the *capacity factor* or *capacity ratio.*

Retention gap: A short piece of deactivated but uncoated column placed between the inlet and the analytical column. A retention gap often helps relieve solvent flooding. It also entrains nonvolatile sample contaminants from on-column injection.

Retention index (I): A uniform system of retention classification according to a solute's relative location between a pair of homologous reference compounds on a specific column under specific conditions. A series of normal straight-chain hydrocarbons, fatty acid esters, or multiring polycyclic aromatic hydrocarbons have been used for the reference compounds. For a solute i that is eluted at t'_{Ri} between two hydrocarbons with chain length z and $z + 1$: $I = 100[z + (\log t'_{Ri} - \log t'_{Rz})/(\log t'_{Rz1} - \log t'_{Rz})]$.

Retention time (t_R): The time between injection and the appearance of the peak

maximum. It is usually measured from the point of injection to the apex of the peak. For asymmetric peaks it should be measured to the center of the mass of the peak. Also called the *total retention time* (IUPAC). See ***retention volume; retention time, adjusted.***

Retention time, adjusted (t'_R): A measure of the retention time adjusted for the void time or unretained peak time: $t'_R = t_R - t_M$ where t_R is the retention time and t_M (or t_0) is the hold-up time, void time, or unretained peak time (that is, the time it takes for a small, unretained compound that completely permeates the pores to be eluted from the chromatographic column).

Retention volume (V_R): The volume of mobile phase required to elute a substance from the column: $V_R = F_c t_R$ or $V_R = V_M + K_D V_S$ where V_M is the void volume, K_D the distribution coefficient, and V_S the stationary phase volume. Also termed the *total retention volume.* See ***retention time.***

Retention volume, adjusted (V'_R): Adjusts the retention volume for the holdup volume (or V_0) where V_R is the retention volume of the peak of interest and V_M is the hold-up or void volume, the volume corresponding to the holdup time: $V'_R = V_R - V_M$

Retention volume, corrected (V_R^0): Corrects the retention volume for the effect of carrier-gas expansion along the column: $V_R^0 = V_R j$

Reversed-phase chromatography: The most frequently used mode in HPLC. It uses low polarity packings such as octadecylsilane or octylsilane phases bonded to silica or neutral polymeric beads. The mobile phase is usually water or water-miscible organic solvents such as methanol or acetonitrile. Elution usually occurs based on the relative hydrophobicity (or lipophilicity) of the solutes; the more hydrophobic, the stronger the retention. The greater the water solubility of the analyte, the less it is retained. There are many variations of reversed-phase LC where various mobile phase additives are used to impart a different selectivity. For example, for the reversed-phase LC of anions, the addition of a buffer and a tetraalkylammonium salt would allow ion pairing to occur and effect separations that rival ion-exchange chromatography. More than 90% of HPLC users employ reversed-phase LC.

Reynolds number (R_e): For flow in a smooth unpacked pipe where $\overline{u}$ is the average velocity (cm/s), d_c is the pipe diameter, η is the viscosity (Pa·s) and ρ is the density (g/cm^3): $R_e = (\overline{u} d_c \rho)/\eta$. The Reynolds number is the ratio of viscous to inertial energy of the moving fluid. At low R_e viscous friction dominates and controls fluid motion, making it slow and steady. In an unpacked tube flow becomes fully turbulent when R_e exceeds 4200. In a packed bed $\overline{u}$ is replaced with the average interstitial velocity and d_c with the average particle diameter. Flow becomes turbulent in a packed bed at R_e above about 10 but is not fully turbulent until R_e exceeds 100–200.

Riffler: A mechanical device used in subdividing solid powder samples into smaller units. Rifflers can be manual or automated. Rifflers will subdivide material samples into two smaller portions by a single pass or further subdivisions can be attained by multiple passes.

Rinse step: In SPE, the rinse (wash) step is the third step in the process. After the sample is loaded, the rinse step is designed to eliminate interferences including various matrix compounds. A solvent (or solvents) or buffer is selected

to remove interferences but not the analytes of interest.

Room temperature (T_o): The room or laboratory temperature can be used as a reference temperature for gas measurements, for example 20 °C or 25 °C.

Round-well plates : 96-well plates that have round-shaped wells resembling 96 small test tubes. See ***96-well plate.***

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Salting-out effect: The use of a high concentration of salt buffer in the mobile phase to cause a low polarity analyte to have a decreased solubility in water and therefore precipitate or come out of solution; most often used for the hydrophobic interaction chromatography of proteins where proteins are first precipitated at high salt concentrations then eluted by gradual dilution using reverse gradient elution. Salting-out is also used in headspace sampling to increase the ionic strength of the sample solution and thereby decrease the solubility of dissolved analytes and increase their headspace concentrations. Can also be used in liquid–liquid extraction; see ***salting-out extraction.***

Salting-out extraction: In this extraction approach, high concentrations of salt in the aqueous phase will cause certain compounds to migrate into an organic phase or perhaps vice-versa; high concentrations of salt also will force normally miscible solvents (such as water and acetonitrile) to become immiscible and be used for further partitioning more polar analytes than could be achieved by an extraction using a non-polar organic solvent. See ***QuEChERS*** or ***salting-out effect***.

Sample capacity: Refers to the amount of sample that can be injected onto a column without overload and loss of column efficiency. Often expressed as grams of sample per gram of packing. Overload is defined as the sample mass injected that will cause the column efficiency to decrease by 10% from its normal value. Sometimes referred to as *sample loading*.

Sample discrimination: The characteristic of systematic change in sample composition according to a specific sample property. For example, a GC inlet may exhibit mass discrimination and admit relatively higher amounts of low-boiling sample components than high-boiling components in the same sample or injection.

Sample division: The process of sample reduction to divide the sample into smaller portions while retaining representative characteristics of the primary sample. See ***sample size reduction.***

Sample loop: Part of an injection valve that delivers an accurate volume of liquid or gas to the column, giving a "slug" injection; loops come in different volumes depending on the needs of the analysis and the size of the column.

Sample pretreatment: Often synonymous with *sample preparation*; the process of manipulating the sample to make it easier to analyze.

Sample size reduction: The process of sample reduction to divide the sample into smaller portions while retaining representative characteristics of the primary sample. See ***sample division.***

Sample tracking: The process of tracking primary, secondary, laboratory, and further samples through the analytical cycle; it is important for chain of custody reasons to be able to ensure that the sample analyzed in the instrument was the original sample collected

at the source; sample tracking can be as simple as writing a sample number on a container but can be more complex, such as using bar-coded vials or radio frequency identification (RFID) tags to automatically keep track of sample flow.

Sampling: The process of collecting a representative sample at the source. Sampling can also refer to further sample division as it more closely approaches the laboratory analysis; it is important to make sure that the final sample analyzed represents a subsample of the original sample without any imposed bias or discrimination.

Sampling error: In statistics, sampling error is incurred when the statistical characteristics of a population are estimated from a subset, or sample, of that population. Because the sample does not include all members of the population, statistics on the sample, such as means and quantiles, generally differ from parameters on the entire population. Because sampling typically is done to determine the characteristics of a whole population, the difference between the sample and population values is considered a sampling error. Exact measurement of sampling error generally is not feasible because the true population values are unknown; however, sampling error can often be estimated by probabilistic modeling of the sample.

Sampling rate: See ***data acquisition rate.***

Sandwich technique: Injection technique in which a sample plug is placed between two solvent plugs in the syringe so as to wash the syringe needle with solvent and obtain better sample transfer into the inlet.

Saturator column: See ***precolumn***.

Scaleability: In going from analytical to preparative chromatography, refers to the reproducibility of results on columns of different internal diameters when using the same particle size and bonded phase; normally a larger diameter column is used to increase capacity; a linear scale-up process minimizes time required to optimize preparative separations.

Scavenger: Special type of solid-phase particle that uses chemical reactions (unlike SPE, which uses molecular interactions) to remove undesired species such as undesired reaction products or excess starting material from an organic synthesis. Scavengers mostly operate on the basis of covalent bonding. Packing materials contain reactive groups that can be used for organic or inorganic species such as catalysts.

Secondary sampling: Refers to the process of taking a representative portion of the primary sample to further reduce its particle size or to prepare a laboratory sample for eventual analysis.

Sedimentation: A technique used for the sizing of resins for ion-exchange chromatography; a broad distribution of beads is placed in a solvent, often water, in a container that is affixed to a stationary surface. Based on particle size and particle density the beads will settle at different velocities into a gradient of sizes and the fraction of interest is removed. Very narrow cuts of particle size can be obtained by sedimentation.

Selectivity: The fundamental ability of a stationary phase to selectively retain substances based on their chemical characteristics, including vapor pressure and polarity. In LC, selectivity is strongly influenced by the mobile-phase composition. In GC, carrier gas has less, if any, impact on chromatographic selectivity.

Selectivity (α): Term replaced by the separation factor. See ***separation factor.***

Selectivity coefficient ($k_{A/B}$): In ion-exchange chromatography, the equilibrium coefficient obtained by application of the law of mass action to an ion exchanger and characterizing the ability of an ion exchanger to select one of two ions present in the same solution. For example, the exchange of Na^+ for H^+ in: $K_{Na/H} = [Na]_S^*[H]_S/[Na]_M[H]_M$

Selectivity triangle: An approach to classify the properties of stationary phases in reversed-phase LC. Results can be represented in a "selectivity triangle" in which the apices of the triangle represent the relative contributions of steric hindrance (χS), hydrogen bonding basicity (χB) and cation exchange capacity (χC) to selectivity. A graphical visualization of the column selectivity allows three-dimensional data to be presented in a two-dimensional space. Provides an informative yet universal approach for phase classification compared to other models. With this model, selection of columns of either equivalent or different selectivity is readily achievable, which should further facilitate the application of reversed-phase LC.

Selectivity tuning: Several techniques for adjusting the selectivity of separations involving more than one column or stationary-phase type. Serially coupled columns and mixed-phase columns can be selectivity-tuned.

Semipreparative chromatography: Refers to preparative LC carried out on an analytical size (4–5 mm i.d.) or a slightly larger (6–10 mm i.d.) column. Normal injection size would be in milligram to low gram amounts.

Sensitivity (*S*): Degree of detector response to a specified solute amount per unit time or per unit volume, often defined by lower limit of detection (LOD). For a concentration-sensitive detector such as a thermal conductivity detector or UV–vis detector: $S = m_{max}/c$ where m_{max} is the peak height and c is the solute concentration in the detector; units of sensitivity for a concentration-sensitive detector that responds in millivolts are mV·mL/g. For mass-flow sensitive detectors such as the flame-ionization detector: $S = m_{max}/W_t$ where W_t is the mass of solute passing through the detector per unit time; the units of S are then expressed as A·s/g or C/g.

Separation: The degree of separation of two peaks in time. See ***separation factor*** (α), ***relative retention, resolution.***

Separation factor (α): The separation factor α expresses the relative retention of two adjacent peaks: $\alpha = t'_{R2}/t'_{R1} = k_2/k_1$ where t'_{R2} is the retention time of the second peak and t'_{R1} is the retention time of the first peak; k_2 and k_1 are the corresponding retention factors.

Separation impedance (*E*): A figure of merit developed by John Knox to compare the efficiency of two chromatographic systems by normalizing for both analysis time and pressure drop: $E = t_R\Delta p/N^2\,\nu(1 + k)$ where t_R is the retention time, Δp is the pressure drop, N is the theoretical plate number, ν is the reduced velocity, and k the retention factor. The lower the value of E, the better the system.

Separation number (*SN*): Separation number, or Trennzahl (*TZ*). A measure of the number of peaks that could be placed with baseline resolution between two sequential peaks, z and z+1, in a homologous series, such as two hydrocarbons: $SN = (t_{R(z+1)} - t_{R(z)})/(w_{h(z+1)} + w_{h(z)})$

Septum: Silicone or other elastiomeric material that isolates inlet carrier flow

from the atmosphere and permits syringe penetration for injection.

Septum purge: Carrier gas is swept across the septum face and out to a separate vent so that material emitted from the septum does not enter the column.

Sequential suppression: Combination of chemical suppression (MSM) and CO_2 suppression (MCS). The background conductivity of carbonate and hydrogencarbonate eluents after suppression is approximately 10–20 µS/cm. This is a result of the dissolved carbonic acid that partially dissociates. The MCS removes the CO_2 from the suppressed eluent and therefore reduces the background even further (typically >1 µS/cm).

Shell particle: See s***uperficially porous particles.***

Sieving: Process of passing a sample of solid particles through a metal or plastic mesh of a uniform cross-sectional area (square opening from 3 µm to 123 mm) to separate particles into uniform sizes; can be performed under wet and dry conditions.

Signal-to-noise ratio (S/N): The ratio of the peak height to the noise level. A detector gives a valid signal if there is some measurable response above the normal background noise; both detector sensitivity and limit of detection are dependent on the level at which the signal can be distinguished. A minimum S/N is equivalent to 2 but for quantitative methods sometimes a higher value is chosen (such as S/N = 6), meaning that the signal is 6 times that of the baseline noise.

Silanol: The Si-OH group found on the surface of silica gel. There are different strengths of silanols depending on their location and relationship to each other and based on the metal content of the silica. The strongest silanols are acidic and often lead to undesirable interactions with basic compounds during chromatography.

Silanophile: A compound that has high affinity for active (acidic) silanol groups on the silica surface. Usually a strongly basic amine.

Silica gel: The most widely used HPLC packing. It has an amorphous structure, is porous, and consists of siloxane and silanol groups. It is used in all modes of LC as a bare packing for adsorption, as the support for LLC or for chemically bonded phases, and, with various pore sizes, as an SEC packing. Microparticulate silicas of 3-, 5-, and 10-µm average particle diameter are used in HPLC. Compared to irregular silicas, in modern analytical HPLC columns, spherical silicas are preferred because of their packing reproducibility and because they have lower pressure drops; sometimes referred to as *silica*. Also used as a gas-solid adsorbent in GC.

Siloxane: The Si-O-Si bond. A principal bond found in silica gel or for a silylated compound or bonded phase. Stable except at high pH values; has little effect on the HPLC separation.

Silylation: The process of reaction of an organochlorosilane or organoalkoxysilane with a compound containing an reactive group. In LC it refers to the process of derivatizing the solute before chromatography to make the solute detectable or to prevent unwanted stationary phase interactions. It can also refer to the process of adding a chemically bonded phase to a solid support or to deactivating the packing to cut down on surface activity.

Simulated distillation (SIMDIS): Boiling-point separation technique

that simulates physical distillation of petroleum products.

Simulated moving bed : A chromatographic system involving a series of columns and valves set up to simulate the countercurrent movement of the mobile and stationary phases to allow for the continuous removal of product and reapplication of sample. A complex form of recycle chromatography used in preparative-scale chromatography.

Single drop microextraction (SDME): A single drop of solvent (1–2 µL) suspended in the headspace can partition volatile analytes into the solvent; the drop can be withdrawn into the syringe and injected into a GC instrument.

Single-ion conductivity (K_i): The single-ion conductivity is proportional to the concentration and the equivalent conductivity of the respective ion.

Size-exclusion chromatography (SEC): Same as ***steric exclusion chromatography.***

Slurry packing: The technique most often used to pack HPLC columns with microparticles. The packing is suspended in a slurry (~10% w/v) and rapidly pumped into the empty column. Special high pressure pumps are used.

Snyder solvent strength parameter (E_0): Solvent strength parameter in adsorption chromatography; the energy of solvent adsorption per unit surface area occupied by the solvent.

Soap chromatography: The earlier name for *ion-pair chromatography*. Long-chain soaps or detergents were used as the mobile phase additives.

Sol gel: Silica gel formed by the aggregation of silica sol; results in type B silica gel with lower surface acidity, lower trace metal, lower surface area and porosity, and higher high pH stability than older type A silica gels.

Solid-phase extraction (SPE): A technique for sample preparation using a solid phase packing (d_p of 20–40 µm) contained in a small plastic cartridge or disk or in a well of a 96-well flow-through plate. The solid stationary phases used are no different than HPLC packings. However, although related to chromatography, the principle is different and is sometimes referred to as *digital chromatography*. The process as most often practiced requires four steps: conditioning the sorbent; adding the sample; washing away the impurities; and eluting the sample in as small a volume as possible with a strong solvent. SPE can be performed in a variety of formats, such as cartridges, disks, pipette tips, and 96-well plates, and in a variety of modes such as reversed phase, ion exchange, and normal phase. It is a widely used sample preparation technique.

Solid support: The same as ***support.***

Solid-core packing: See ***superficially porous particles.***

Solid-phase microextraction (SPME): A technique in which a small polymer-coated solid fiber is placed into a solution or above the headspace of a solid or liquid sample; analytes will diffuse into the coating until equilibrium is established; for GC, the fiber containing the sorbed sample is transferred to the GC and the trapped analytes are thermally desorbed into the column. In HPLC, solvent is used to rinse the sorbed analytes for eventual injection into the LC column; less popularly used in LC than in GC.

Solid-phase trapping: The use of an SPE cartridge or packed column to trap specific analytes that flow through the device; the packing material is chosen to selectively retain the analytes of interest

and let other compounds pass through unretained.

Solute: The dissolved component of a mixture that is to be separated in the chromatographic column. May be referred to as the *analyte.*

Solvent: The liquid used to dissolve a sample for injection into a chromatography column or CE capillary; sometimes refers to the mobile phase used in LC.

Solvent demixing: Occurs when two solvents with very different strengths (A = weak solvent and B = strong solvent) are used with unmodified silica or alumina; the strong solvent (B) will be preferentially adsorbed by the active surface of the stationary phase until it is saturated; until this occurs, the weak solvent (A) will be enriched (demixed) as it travels down the column; eventually when the entire column is saturated with B, this solvent will elute mixed with A at the initial strength and sample components are eluted with the sudden change in solvent strength.

Solvent effect: A solute-profile sharpening technique used with splitless and on-column injection. Condensed solvent in the column during and shortly after injection traps volatile solutes into a narrow band. See also ***retention gap.***

Solvent exchange: The process of exchanging one solvent that may not be compatible with the analysis method for a solvent that is more compatible. In some cases, evaporation is used to remove a volatile solvent and the sample is reconstituted in a different solvent.

Solvent flooding: A source of peak-shape distortion caused by excessive solvent condensation inside the column during and after splitless or on-column injection.

Solvent flushing: A column rinsing technique that may remove nonvolatile sample residue and partially restore column performance. Some stationary phases may be damaged by solvent rinsing or flushing.

Solvent selectivity: Ability of a solvent to influence selectivity; there is solvent strength selectivity where a change in solvent strength (say from 5% B to 10% B) will change band spacing or solvent-type selectivity where change from methanol to acetonitrile as a reversed-phase organic modifier will change band spacing.

Solvent selectivity triangle: A useful guide for choosing among different solvents for the purposes of changing band spacing; solvent selectivity is dependent on dipole moment, acidity, and basicity of the solvent molecule. For details, see L.R. Snyder, P.W. Carr, and S.C. Rutan, *J. Chromatogr. A* **656,** 537–547 (1993).

Solvent strength (*S*): Refers to the ability of a solvent to elute a particular solute or compound from a column. Described for HPLC by Lloyd Snyder for linear elution adsorption chromatography on alumina, solvents were quantitatively rated in an eluotropic series; *S* varies with modifier type, stationary phase, and temperature. Less extensive data are available for silica and carbon adsorbents.

Sonication: The use of ultrasound to create vigorous agitation at the surface of a finely divided solid material. The direct method uses a specially designed inert acoustical tool (a horn or probe, called a *sonotrode*) placed in sample–solvent mixture. In the indirect method, a sample container is immersed in an ultrasonic bath with solvent and subjected to ultrasonic radiation. Dissolution is aided by the ultrasonic process. Heat can be added to increase the rate of extraction. The method is safe and rapid and is best for coarse, granular material. With the

indirect method, multiple samples can be done simultaneously.

Sorb: The process of being retained by a stationary phase when the retention mechanism — adsorption, absorption. partitioning — is not clear.

Sorbent: Refers to a packing used in adsorptive chromatography LC. Common sorbents are polymers, silica gel, alumina, titania, and zirconia and chemically modified materials.

Soxhlet extraction: A well accepted technique for the extraction of compounds from a solid sample; the sample is placed in a disposable porous container (thimble); constantly refluxing fresh condensed solvent flows through the thimble and dissolves analytes that are continuously collected in a boiling flask; special glassware called a *Soxhlet extractor* is designed to perform the extraction unattended.

Specific surface area: The surface area of an LC packing based on measurement by an accepted technique such as the BET method using nitrogen adsorption.

Spherical packing: Refers to spherical solid packing materials. In analytical HPLC spherical packings generally are preferred over irregular particles but in preparative work irregular particles are often used because of their lower cost.

Spin column: A small column that usually contains a packing material for sample cleanup or isolation; the sample is added to the column, which has a selective packing material; the column, in turn, fits into a small collector tube that is placed in a centrifuge, and the liquids in the tube are separated by centrifugation. Spin tubes are very popular in handling biological samples for isolating DNA, RNA, and other biocompounds of interest.

Spin filter: Similar to a spin column but instead of the column packing a membrane filter is used; the purpose of the filter is to remove particulates.

Spin tube: See ***spin column.***

Split injection: An injection technique for GC where only a portion of the sample is directed to the column. The ratio of the vented volume to the injected volume is called the *split ratio,* which has typical values of 100:1, 50:1, and so on. Split injection tries to avoid overloading the column; it ensures a representative sample reaches the column. The technique is simple and rugged and protects the column. However, sample discrimination is possible; splitless injections are usually performed automatically.

Split ratio (*s*): The ratio of the sample amount vented to the sample amount entering the column in split injection. Higher split ratios place less sample on the column. Usually measured as the ratio of total inlet flow to column flow: $s = (F_s + F_c)/F_c$

Split vent flow rate (F_s): Carrier gas flow rate from the split vent, measured at room temperature and pressure.

Splitless injection: Derivative of split injection. During the first 0.5 to 4 min of sampling the sample is not split, and enters only the column. Splitting is restored afterwards to purge sample remaining in the inlet. Up to 99% of sample enters the column. Ensures higher sensitivity than split injections but flashback can occur and a higher possibility of sample degradation is possible as a result of longer residence time in the hot injection port.

Square-well plates: 96-well plates that have square-shaped wells instead of the normal round-bottom wells.

Stagnant mobile phase: The fraction of the mobile phase contained with the pores of the particle.

Standard addition: Process used to improve quantitation; necessary to have a pure standard of known concentration. An unknown concentration of sample is first injected to give a peak area; then to the unknown concentration a measured amount of pure compound is added. As a result of the new peak area, one can determine the original concentration. An alternative procedure is to add a constant amount of unknown concentration to a series of standards of pure substances and to plot the peak areas obtained against the known concentrations of the original standards. The slope of the line obtained gives the concentration of the unknown.

Standards: A sample that contains known quantities of the compounds of interest. Standards are used to help identify sample peaks by comparing the time in which they are eluted to the retention times obtained through the injection of the sample under the same conditions. For quantitation, external standards are compounds that are used to construct calibration curves of detector output (peak area or peak height) versus concentration; the concentration of unknowns is determined by fitting the detector output to the calibration curve. Internal standards are compounds of known concentration with different retention times that are added to the sample and relative detector responses between the internal standard and the unknown are compared in order to quantitatively measure unknown compounds.

Static coating: A technique for stationary-phase deposition in open-tubular columns. The column is filled with a solution of stationary phase and one end sealed. A vacuum, heat, or both are applied to the open end. As the solvent evaporates, a thin uniform film of stationary phase is left behind.

Stationary phase: The immobile phase involved in the chromatographic process. The stationary phase in LC can be a solid, a bonded, immobilized, or coated phase on a solid support, or a wall-coated phase. The stationary phase used often characterizes the separation LC mode. For example, in LC, silica gel is used in adsorption chromatography, whereas an octadecylsilane bonded phase is used in reversed-phase chromatography. In GC, liquid or polymeric stationary phases are used for liquid–liquid partitioning separations, and porous-polymer, silica, alumina, or molecular sieve packings are used for adsorption and molecular size-based separations.

Stationary phase film thickness (d_f): The average thickness of the stationary-phase film coated on the walls of an open-tubular (capillary) GC column. Most open-tubular GC columns have film thicknesses of 0.1–5 μm.

Stationary zone: To be distinguished from the stationary phase. The stationary zone includes the stagnant mobile phase and the chromatographically active stationary phase.

Step gradient: See ***stepwise elution***.

Stepwise elution: Use of eluents of different compositions during the chromatographic run. These eluents are added in the stepwise manner with a pump or by a selector valve. Gradient elution is the continuous version of changing of solvent composition.

Steric exclusion chromatography (SEC): A major mode of LC in which samples are separated by virtue of their size in solution. Also known as *size exclusion chromatography, gel permeation chromatography, gel filtration chromatography,* or *gel chromatography.* SEC is most often

used for polymer separation and characterization, the separation of proteins, and the desalting of biological samples.

Sterically protected bonded phase: Bonded phase that has sterically protecting bulky functional groups such as isopropyl and isobutyl surrounding siloxane covalent surface bond; prevents attack on siloxane bond and prevents catalyzed hydrolysis and loss of bonded phase at low pH (< 3).

Stir-bar sorbent extraction (SBSE): Principle similar to solid-phase microextraction (SPME) but instead of a coated fiber a polymer-coated stir bar is used, greatly increasing the surface area, thus providing higher capacity and greater mass sensitivity. Similar to SPME, equilibration requires tens of minutes. For GC, a special thermal desorption unit is needed to handle the stir bar; in LC, the stir bar is usually rinsed off-line.

Straight phase chromatography: Same as ***normal-phase chromatography.***

Strong anion exchanger: Anion-exchange packing with strongly basic ionogenic groups (for example, tetraalkylammonium).

Strong cation exchanger: Cation-exchange packing with strongly acidic ionogenic groups (for example, sulfonic).

Strong solvent: In general, refers to a solvent which is a good solvent for a chemical compound; in chromatography, refers to the mobile phase constituent that provides a higher solvent strength that causes an analyte to elute more quickly from the column; in a water-acetonitrile binary solvent system for reversed-phase liquid chromatograhy, acetonitrile would be considered to be the strong solvent.

Sub-2-µm packing: A term that refers to the use of porous packings below 2 µm average particle diameter; current products vary from 1.5 to 2.0 µm.

Sulfonyl cation exchanger: A strong cation-exchange functionality found in resin-based packings; usually propyl-SO_3H; may come in other cationic forms such as sodium, ammonium, silver, and calcium.

Sulfur chemiluminescence detection (SCD): Detection method that responds to sulfur-containing compounds by generating and measuring light from chemiluminescence.

Supercritical fluid: The defined state of a compound, mixture, or element above its critical pressure and critical temperature.

Supercritical fluid chromatography (SFC): A technique that uses a supercritical fluid as the mobile phase. The technique has been applied to the separation of substances which cannot be handled effectively by LC (because of detection problems) or GC (because of the lack of volatility). Examples are separations of triglycerides, hydrocarbons, and fatty acids. GC detectors and HPLC pumps have been used together in SFC.

Supercritical fluid extraction (SFE): Uses supercritical fluid, most often carbon dioxide alone or containing a small percent of organic modifier for more polar analytes, to extract analytes from solid materials; supercritical fluid has the diffusivity of a gas and the solvent power of a liquid; requires a special SFE unit where the pressure and temperature can be precisely controlled; analytes are collected in a cold trap, on an adsorbent or in a liquid; a "green" extraction technique.

Superficially porous particles (SPPs): Same as ***porous-layer bead***. Recently there has been a revival of superficially porous particles based on smaller

particles (1.3–5.0 µm) with thicker porous shells (0.3–0.6 µm); such particles give similar or better performance than sub-2-µm particles.

Superheated water extraction: Water is heated well above its boiling point in a closed pressurized system; heating changes dielectric constant and increases the solvating power such that it becomes "organic-like." It is a "green" method for extracting organic analytes from solid matrices.

Support: Refers to solid particles. Support can be naked, coated, or have a chemically bonded phase. The solid support doesn't contribute to the liquid–liquid chromatographic process but is active for adsorptive processes.

Support-coated open-tubular column (SCOT): A capillary column in which stationary phase is coated onto a support material that is distributed over the column inner wall. A SCOT column generally has a higher peak capacity than a wall-coated open tubular column (WCOT) with the same average film thickness. See ***wall-coated open tubular column WCOT.***

Supported liquid extraction (SLE): A technique based on the principles of liquid–liquid extraction where the aqueous phase is supported on a bed of highly purified, high surface area diatomaceous earth (in a tube, cartridge, or 96-well format); this aqueous phase may be buffered and may contain the sample to be partitioned; the organic phase is then percolated through the packed bed allowing for intimate contact with the dispersed aqueous phase. The effluent collected at the exit of the column contains the extracted analytes; compared to LLE, the SLE technique is miniaturized, easily automated, and provides excellent extraction efficiency.

Suppression: Method to reduce the background signal before detection (see ***electrochemical suppression***, ***chemical suppression***, and ***sequential suppression***). Typically used together with conductivity detection.

Suppressor column: In ion chromatography, refers to the column placed after the ion-exchange column. Its purpose is to remove or suppress the ionization of buffer ions so that sample ions can be observed in a weakly conducting background with a conductivity detector; sometimes rather than a column, membrane suppressors are used.

Surface area: In an adsorbent, refers to the total area of the solid surface as determined by an accepted measurement technique such as the BET method, which uses nitrogen adsorption. The surface area of a typical porous adsorbent such as silica gel can vary from 100 to 600 m^2/g.

Surface coverage: Usually refers to the mass of stationary phase per unit area bonded to an LC support. Often expressed in micromoles per square meter of surface. Sometimes %C is given as an indicator of surface coverage.

Surrogate samples: A pure analyte that is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amounts before extraction and is measured with the same procedures used to measure other sample components. A surrogate behaves similarly to the target analyte and is most often used with organic analytical procedures. The purpose of a surrogate analyte is to monitor method performance with each sample.

Swelling or shrinking: Process where resins and gels increase (or decrease) their volume because of their solvent environment. Swelling is dependent on the

degree of crosslinking; low cross-linking resins will swell and shrink more than highly cross-linked resins. If swelling occurs in a packed column blockage or increased back pressure can occur. In addition, column efficiency can be affected.

Syringe filter: A small plastic holder containing a membrane filter that has Luer-lock fittings at both the top and the bottom so that it can be affixed to a syringe (which also has a Luer-lock fitting) to pass a sample through the filtration media. Syringe filter diameters can range up to 90 mm.

System dispersion: The contribution to band broadening outside of the column itself; it generally refers to the instrumental contributions as well as other extra column contributions. With newer high efficiency columns, decreasing the system dispersion contributions will result in better chromatographic performance.

System peak: The system peak is the peak of the eluent ion. There is no possibility of quantifying that peak. It is an unwanted peak in the chromatogram. A possible explanation of the system peak: Because of the sample injection, the ion exchange equilibrium of the eluent ions get disturbed. The re-equilibration process yields in this additional peak. It appears in suppressed and nonsuppressed IC, but it is pretty small with suppression. Using sequential suppression minimizes the system peak.

T

Tailing: The phenomenon where the normal Gaussian peak has an asymmetry factor greater than one. The peak will have an extended trailing edge. Tailing is caused by sites on the packing that have both a stronger-than-normal retention for the solute and slower desorption kinetics. A typical example of a tailing phenomenon would be the strong adsorption of amines on the residual silanol groups of a low coverage reversed-phase packing at intermediate pH values. Tailing can also result from injecting an excessive mass or sample, from badly packed columns, from excessive extracolumn volume, poor fittings, and excessive detector volume, or slow detector response. Tailing peaks show an asymmetry factor greater than 1.0; see ***asymmetry factor.***

Tailing factor: U.S. Pharmacopeia measure of peak asymmetry defined as the ratio of the peak width at 5% of the apex to two times the distance from the apex to the 5% height on the short time side of the peak. Greater than unity for tailed peaks. See Figure 1 and ***asymmetry factor.***

TCDD: Tetrachlorodibenzo-*p*-dioxin.

TCEP: Stationary phase for GC: tris-cyanoethoxypropane.

Tedlar bags: Used for grab sampling of air or other gases; Tedlar (Dupont) sampling bags are a whole-air sampling device for high-level volatile organic compounds (VOCs) and permanent gases. Several EPA, NIOSH, and OSHA methods exist for bag sampling for a variety of applications: stationary sources emissions, workplace atmospheres, ambient, indoor air quality, and breath analysis. The unique design of these sample bags incorporates the sampling septum directly in the valve (polypropylene or stainless steel construction), providing easier use and lighter weight than other styles.

Ternary mobile phase: Mobile phase consisting of a mixture of three individual solvents or buffers or both.

Theoretical plate: A hypothetical entity inside a column that exists by analogy to a multiplate distillation column. As solutes migrate through the column they

partition between the stationary phase and the carrier gas. Although this process is continuous, a stepwise model is often visualized. One step roughly corresponds to a theoretical plate.

Theoretical plate height (*H*): The distance along a chromatographic column that corresponds to a single theoretical plate. $H = L/N$ where L is column length and N is the number of theoretical plates. A carryover from distillation theory; a measure of efficiency of a column. For a typical well packed HPLC column, H should be about 2–3 d_p for 5-µm particles, usually in the range of 0.01–0.03 mm; modern superficially porous and sub-2-µm particles sometimes show plate heights of less than 2 d_p. In open-tubular column GC, H should be between 0.5–2 times the column inner diameter. HETP is a deprecated term for the plate height. The combined van Deemter–Golay equation gives the theoretical plate height for a chromatography column: $H = A + B/\overline{u} + \overline{u}(C_M + C_S)$ where A is the contribution due to eddy diffusion and multipath flow and B is the contribution from longitudinal solute diffusion in the mobile phase. The C terms are related to the effects of diffusion on mass transfer; C_M in the mobile phase and C_S in the stationary phase. See ***A term, B term, C term, Golay equation,*** and ***van Deemter equation.***

Theoretical plate height, minimum (H_{min}): The minimum of the van Deemter curve that results from a plot of H versus u (LC) or H versus $\overline{u}$ (GC). This value represents the most theoretical plates that can be obtained for a certain column and mobile phase system. Usually occurs at excessively slow flow rates. Also known as the optimum plate height. For well-packed columns it is typically 2–3 times the particle diameter; for open-tubular columns 0.5–2 times the inner column diameter and, ignoring stationary-phase contributions to band broadening: $H_{min} = (d_c/2)((1 + 6k + 11k^2)/(3(1 + k)^2))^{1/2}$

Theoretical plate height, reduced (*h*): Used to compare efficiencies of different columns. A reduced plate-height value of 2 or less at the optimum velocity is considered to be a well-packed column. For packed columns: $h = H/d_p$. For open-tubular columns: $h = H/d_c$

Theoretical plate number (*N*): The number of theoretical plates measured in a column. A concept described by Martin and Synge. Relates chromatographic separation to the theory of distillation. The length of column that corresponds to a single theoretical plate relating to this concept is called the *plate height* or *height equivalent to a theoretical plate*. The larger the plate number, the more theoretical plates the column possesses. A typical well-packed HPLC column with a 5-µm porous packing in a 15-cm column of 4.6-mm i.d. should show 10,000–12,000 plates, which is the same number of plates for a 5-cm column of the same internal diameter packed with sub-2-µm particles or superficially porous particles. A typical 25-m, 0.25-mm i.d. open-tubular GC column with a thin stationary-phase film of 0.25 µm or less should exhibit 50,000 theoretical plates or more. The theoretical plate number is calculated from a chromatogram as follows: $N = 16(t_R/w_b)^2 = 5.54(t_R/w_h)^2$ where w_b is the width at the peak base and w_h is the peak width at half-height. See ***theoretical plate height.***

Theoretical plates, effective (N_{eff}): The true number of theoretical plates in a column. The number of effective theoretical plates corrects theoretical plates (N) for hold-up volume: $N_{eff} = 16(t'_R/w_b)^2$ where t'_R is the adjusted retention time and w_b

is the peak width at base. It is a better figure of merit than simple plate number when comparing devices of very different geometries and phase ratios; sometimes referred to as *effective plate number*.

Theoretical plates, required (N_{req}): Number of theoretical plates required to yield a particular resolution (R) at a specific peak separation (α) and retention factor (k): $N_{req} = 16R^2 (\alpha/(\alpha - 1))^2 ((k + 1)/k)^2$

Thermal desorption: The use of heat to desorb analytes from SPME fibers, an SBSE bar, or solid matrices placed in a thermal desorption tube.

Thermal extraction: Uses high temperatures (below pyrolysis temperatures) to extract stable analytes from porous solid matrices; samples are placed in thermal desorption tubes just as in ***thermal desorption.***

Thermal-conductivity detection (TCD): A thermal-conductivity detector measures the differential thermal conductivity of carrier gas and reference gas flows. Solutes emerging from a column change the carrier-gas thermal conductivity and produce a response. TCD is a universal detection method with moderate sensitivity.

Thermally tuned tandem column chromatography (T3C): A form of LC in which two columns with distinctly different selectivities are placed in tandem and operated at two different temperatures so as to optimize the resolution and/or speed of analysis. A common eluent is used in both columns and the entire sample passes through both columns and is detected with a single detector. It is not a two-dimensional technique in that each sample component gives only a single peak.

Thermionic specific detection (TSD): See ***nitrogen–phosphorus detection.***

Time-integrated sampling: In gas sampling, to obtain a more representative sample requires time-integrated sampling. A flow restrictor is used to spread the sample collection flow over a specific time period to ensure an "average" composited or time-weighted average (TWA) sample. A TWA sample will accurately reflect the mean conditions of the ambient air in the environment and is preferred when, for regulatory or health reasons, a typical exposure concentration is required for a situation that may have high variability, as in an occupational setting.

Titania: TiO_2, is an uncommon adsorbent used in adsorption chromatography; also used as an SPE sorbent primarily for removal of phosphorous-containing compounds such as phospholipids.

TMS: Trimethylsilyl (a chemical derivative). In LC, the TMS group is frequently found on endcapped silica gel-based columns.

Tortuosity (tortuousity factor) (ω): A property of a packed column that controls the inhibition of longitudinal diffusion of the solute as it diffuses along the column axis. The B term in the van Deemter equation is proportional to the tortuousity.

Total mobile-phase volume (V_t): In SEC the total volume of mobile phase in the column. The same as V_M. Also known as the *totally included volume*.

Total permeation volume (V_p): The retention volume on an SEC packing where all molecules smaller than the smallest pore will elute. In other words, at V_p all molecules totally permeate all of the pores and are eluted as a single peak.

Total porosity (ε_T): The ratio of the total volume of mobile phase in the column to the total column volume: $\varepsilon_T = V_M/V_c = \varepsilon_e + \varepsilon_i(1 - \varepsilon_e)$

Totally porous packing: A stationary phase that is a porous matrix. Solutes penetrate the porous to interact with the stationary phase.

Trace enrichment: Technique where trace amounts of compounds are retained on an HPLC or precolumn packing out of a weak mobile phase or solution and then are eluted by the addition of a stronger mobile phase in a concentrated form. The technique has been most successfully applied in the concentration of trace amounts of hydrophobic compounds (for example, polynuclear aromatic hydrocarbons) out of water using a reversed-phase packing. A strong solvent such as acetonitrile serves to elute the enriched compounds.

Trapping: Process of using a solid material (such as silica gel, polymer, or inorganic sorbent) or liquid solution to physically or chemically retain solutes of interest from a diluted stream of liquid or gas. Frequently used to concentrate analytes for more sensitive analysis.

Trennzahl (*TZ*): See ***separation number.***

Triethyl amine: A very common additive used to block silanol groups in reversed-phase LC when separating basic analytes.

Trifluoroacetic acid: A very common mobile phase additive in reversed-phase LC for peptides and proteins. Also a derivatization reagent for amines and carboxylic acids.

Tryptic digestion: A method of selectively and reproducibly dissecting peptide chains of proteins to yield a characteristic pattern of smaller units that allows analysis of the parent protein by gradient elution reversed-phase liquid chromatography.

Turbulence: In fluid dynamics, turbulence or ***turbulent flow*** is a flow regime characterized by chaotic and stochastic property changes. Flow in which the kinetic energy dies out as a result of the action of fluid molecular viscosity is called *laminar flow*. Although there is no theorem relating the nondimensional Reynolds number (R_e) to turbulence, flows at Reynolds numbers larger than 4200 are typically (but not necessarily) turbulent, whereas those at low Reynolds numbers usually remain laminar.

Turbulent flow: A form of fluid motion in which the flow ceases to be smooth and steady, and becomes chaotic and fluctuates with time. It is characterized by a pressure drop significantly higher than that which would be extrapolated from the laminar region to achieve the same volumetric flow rate.

Turbulent flow chromatography: Chromatography performed at very high linear velocities with large particles, if present, under conditions using high Reynolds numbers. At these conditions the H versus u curves show a decrease in H with increase in u. Turbulent flow chromatography can be used for separation or sample preparation.

Two-dimensional chromatography: A procedure in which part or all of the separated sample components are subjected to additional separation steps. This can be done by conducting a particular fraction eluted from the first column into a second column or system having a different separation characteristic. It includes techniques such as two-dimensional thin-layer chromatography using two eluent systems, where the second eluent is applied after rotating the plate through 90°. This also includes LC or GC followed by GC, or one LC mode followed by a different mode — for example, reversed-phase LC followed by SEC.

Two-dimensional electrophoresis: Two-dimensional gel electrophoresis, abbreviated as 2DE or 2D electrophoresis, is a form of gel electrophoresis commonly used to analyze proteins. Mixtures of proteins are separated by two properties in two dimensions on 2D gels. 2D electrophoresis begins with 1D electrophoresis but then separates the molecules by a second property in a direction 90° from the first. In 1D electrophoresis, proteins (or other molecules) are separated in one dimension, so that all the proteins will lie along a lane; the molecules are spread out across a 2D gel. Because it is unlikely that two molecules will be similar in two distinct properties, molecules are more effectively separated in 2D electrophoresis than in 1D electrophoresis.

Type A silica: Silica gel formed by gelling soluble silicates; generally higher acidity, higher surface area and porosity, more trace metals, poorer high-pH stability than Type B silicas.

Type B silica: See ***sol gel.***

U

Ultrahigh-pressure liquid chromatography (UHPLC): Ultrahigh-pressure liquid chromatography is often used loosely for any separation performed at pressures greater than provided by conventional pumps (400 bar); original meaning was for separations in the 20,000 psi+ range.

Ultrafiltration: Variety of membrane filtration in which hydrostatic pressure forces a liquid against a semipermeable membrane. Suspended solids and high-molecular-weight solutes are retained, and water and low-molecular-weight solutes pass through the membrane. This separation process is used for purifying and concentrating macromolecular (10^3–10^6 Da) solutions, especially protein solutions. Ultrafiltration is not fundamentally different from microfiltration, nanofiltration or gas separation, except in terms of the size of the molecules it retains. Ultrafiltration is applied in cross-flow or dead-end mode and separation in ultrafiltration undergoes concentration polarization.

Ultrasonic sieving: Used for the acceleration of sieving processes alternatively or complementary to the classical low frequency vibrators. Especially useful for very fine powders where ultrasound is often the only possibility to enable the sieving process at all.

Ultrasonication: The irradiation of a liquid sample with ultrasonic (>20 kHz) waves resulting in agitation. Sound waves propagate into the liquid media result in alternating high-pressure (compression) and low-pressure (rarefaction) cycles. During rarefaction, high-intensity sonic waves create small vacuum bubbles or voids in the liquid, which then collapse violently (cavitation) during compression, creating very high local temperatures; several regulatory methods for environmental samples (for example, soils or solid waste) specify ultrasonication.

USP categories for chromatographic columns: United States Pharmacopeia characterizes columns for use in their HPLC methods by an "L" designation: L1 = octadecylsilane, L7 = octylsilane, L8 = aminopropyl, and so on. For GC columns, a "G" designation is used: G1 and G2 are dimethylpolysiloxane columns, G3 is a 50% phenylmethyl–polysiloxane column, G16 is a polyethylene glycol column, and so on.

UV–vis detection: The absorbance of light is the signal for measuring the chromatogram. There are four different ways of applying UV–vis detection in IC: direct UV–vis, indirect UV–vis, UV–vis after postcolumn reaction, and UV–vis after precolumn reaction.

V

Vacancy chromatography: Technique where a mobile-phase additive causes a positive detector signal output. When a solute is eluted from the column, it dilutes the signal and yields a negative peak ("a vacancy"). The technique has been mostly been applied to single column ion chromatography where mobile phases such as citrate and phthalate buffers absorb in the UV. When a nonabsorbing anion is eluted it dilutes the UV-absorbing background and causes a negative peak; the detector output leads are usually reversed so that the chromatogram looks normal. The technique has also been used in CE for detection.

Vacuum compensation: Method of carrier-gas pressure control in GC with the column exit at mass-selective detector vacuum levels. Enabling vacuum compensation adjusts the column inlet pressure to maintain a set flow or velocity when the column exit is not at room pressure.

Vacuum filtration: Using a vacuum to help pull liquids through a filter; especially useful for viscous liquids or very fine, low porosity filters.

Vacuum manifold: A manifold designed for SPE cartridges and SPE disks that uses a vacuum to pull liquids through the packed beds; pressurized manifolds are also available. Vacuum manifolds can process multiple samples from ranging from 8 to 24 at a time.

van Deemter equation: An equation used to explain the band broadening in chromatography. The equation represents the height of a theoretical plate (H) and has three terms. The A term is used to describe eddy dispersion (diffusion) that results from axial velocity heterogeneity. The B term is for the contribution from molecular diffusion or longitudinal diffusion for the solute while passing through the column. The C term is the contribution from interphase mass transfer and allows for the finite rate of transfer of the solute between the stationary phase and mobile phase. In its simplest representation it is expressed as follows: $H = A + B/u + C\,u$. The van Deemter equation applies to packed columns both for LC and for GC. The related Golay equation applies to open-tubular or capillary GC columns. See ***Golay equation.***

Velocity (u): The same as ***velocity, linear.***

Velocity, average linear ($\bar{u}$): The average speed at which a molecule of GC carrier gas or LC liquid mobile phase passes through a column: $\bar{u} = L/t_M$ where L is the column length and t_M is the hold-up or unretained peak time.

Velocity, column outlet (u_o): In GC, the carrier gas velocity at the column outlet. Equal to the average carrier gas velocity divided by the compressibility correction factor: $u_o = \bar{u}/j$. The carrier gas expands as it pass through the column from the inlet to the outlet pressure, which causes the local carrier-gas velocity to increase along the column. The outlet velocity is always greater than the average velocity. See ***compressibility correction factor.***

Velocity, interstitial (u_e): The actual velocity of the eluent as it moves through the column flowing around

the particles: $u_e = F/(A_c\ \varepsilon_c)$. The interstitial velocity is the basis for computation of the reduced velocity.

Velocity, linear (u): The velocity of the mobile phase moving through the column. Expressed in cm/s. In LC it is directly related to column flow rate by the cross-sectional area of the column and is determined by dividing the column length (L) by the retention time of an unretained compound: $u = L/t_M$. In GC, the speed at which carrier gas moves through the column usually is expressed as the average linear velocity to account for carrier-gas compressibility. See ***hold-up time; velocity, average linear; velocity, column outlet.***

Velocity, mobile phase (u_M): The velocity at which the liquid mobile phase percolates through the bed of particles in an LC column: $u_M = L/t_M$. See ***velocity, linear; velocity, average linear.***

Velocity, optimum linear (U_{opt}): The mobile-phase velocity corresponding to the minimum theoretical plate height, ignoring stationary-phase contributions to band broadening. In open-tubular GC: $u_{opt} = 8\ (D_G/d_c)\ ((3(1 + k)^2)/(1 + 6K + 11k^2))^{1/2}$

Velocity, reduced (ν): Along with the reduced plate height, the reduced velocity is used to compare different chromatographic columns. It relates the solute diffusion coefficient (D_M) in the mobile phase to the particle size of the column packing (d_p): $\nu = u\ d_p/D_m$ where u is the interstitial mobile-phase linear velocity in packed columns, or the average carrier-gas linear velocity in GC. For open-tubular columns, the column internal diameter is used instead of the particle diameter.

Velocity, superficial (u_s): The hypothetical velocity the mobile phase would have if the same column were operated unpacked but with the same flow rate: $u_s = F/A_c$

Velocity, zone (u_z): The velocity of travel of the solute zone: $u_z = u/(1 + k) = L/t_R$

Viscosity (η): Also referred to as *mobile phase viscosity*. The viscosity of the mobile phase varies with the temperature of the column. Column back pressure is directly proportional to solvent viscosity. Low-viscosity mobile phases generally give better efficiency than less viscous ones because diffusion coefficients are inversely related to solvent viscosity. For example, in reversed-phase LC, column efficiency is higher with acetonitrile as an organic modifier than with isopropanol which is more viscous. In GC, the viscosity of the gaseous mobile phase increases with temperature, which causes the carrier-gas flow rate and linear velocity to decrease during temperature programming if the inlet pressure is held constant. Different GC carrier gases such as nitrogen, helium, or hydrogen have different viscosities.

Void: The formation of a space or gap, usually at the head of the column, caused by a settling or dissolution of the column packing. A void in the column leads to decreased efficiency and loss of resolution. Even a small void can be disastrous for small particle microparticulate columns. The void can be removed sometimes by filling it with glass beads or the same porous packing used in the remainder of the column.

Void time: See ***hold-up time.***

Void volume: See ***hold-up volume.***

Volume, liquid phase: See ***volume, stationary phase.***

Volume, mobile phase (V_G or V_M): For wall-coated open-tubular columns

(WCOT), ignoring the stationary phase film thickness ($d_f = 0$): $V_G \approx L(\pi d_c^2)/4$

Volume, stationary phase (V_L or V_S): Volume of the liquid stationary phase contained in the column. The ratio of the mobile-phase volume to the stationary liquid-phase volume is the phase ratio of a GC column. See ***phase ratio.***

W

Wall effect: The consequence of a looser packing density near the walls of a rigid HPLC column. The mobile phase has a tendency to flow slightly faster near the wall because of the increased local permeability. The solute molecules that happen to be near the wall are carried along faster than the average of the solute band and, consequently, band spreading results and there is a loss of column efficiency.

Wall-coated open-tubular column (WCOT): An open-tubular (capillary) GC column in which a uniform stationary phase film is coated directly onto the column wall. See also ***porous-layer open tubular (PLOT) column, support-coated open-tubular column (SCOT).***

Wash step: See ***rinse step.***

Water dip: Indicates the hold-up time in suppressed IC. Usually a negative peak that corresponds to the volume of sample water. The area depends on the difference in concentration of the eluent anions between eluent and sample. The water dip is large if the sample is almost ultrapure water. If the sample is diluted with eluent there will be almost no water dip.

Weak anion exchanger: Anion-exchange packing with weakly basic ionogenic groups (for example, amino or diethylaminoethyl).

Weak cation exchanger: Cation-exchange packing with weakly acidic ionogenic groups (for example, carboxyl).

Weak solvent: In general, refers to a solvent that is a poor solvent for a particular chemical compound; in chromatography, refers to the mobile phase constituent that provides a low solvent strength and causes an analyte to be eluted more slowly from the column. In a water–acetonitrile binary solvent system for reversed-phase LC, water would be considered the weak solvent; in a binary solvent eluent would normally be the "A" solvent.

Wide-bore open-tubular column (WBOT): Open-tubular (capillary) GC column with a nominal inner diameter d_c of ≥530 µm.

Wilke-Chang equation: A semiempirical equation used to estimate diffusion coefficients in liquids as a function of molecular size of solute and solvent viscosity.

XYZ

Xerogels: Gels used in SEC that will swell and shrink in different solvents; also refers to silica-based packings that are prepared from acidification of soluble silicates to give a amorphous, high surface, high porosity, rigid particle.

Zero dead volume: Any fitting or component in which all of the volume is swept by the eluent. See ***dead volume.***

Zirconia: Porous zirconium oxide; used as a chromatographic sorbent usually coated or bonded with polymeric organic phase.

Zone: See ***band.***

Zwitterionic packing: A packing material for HPLC that carries both positive and negative charges on its surface; zwitterionic packings are useful in the HILIC mode.

Zwitterions: Compounds that carry both positive and negative charges in solution.

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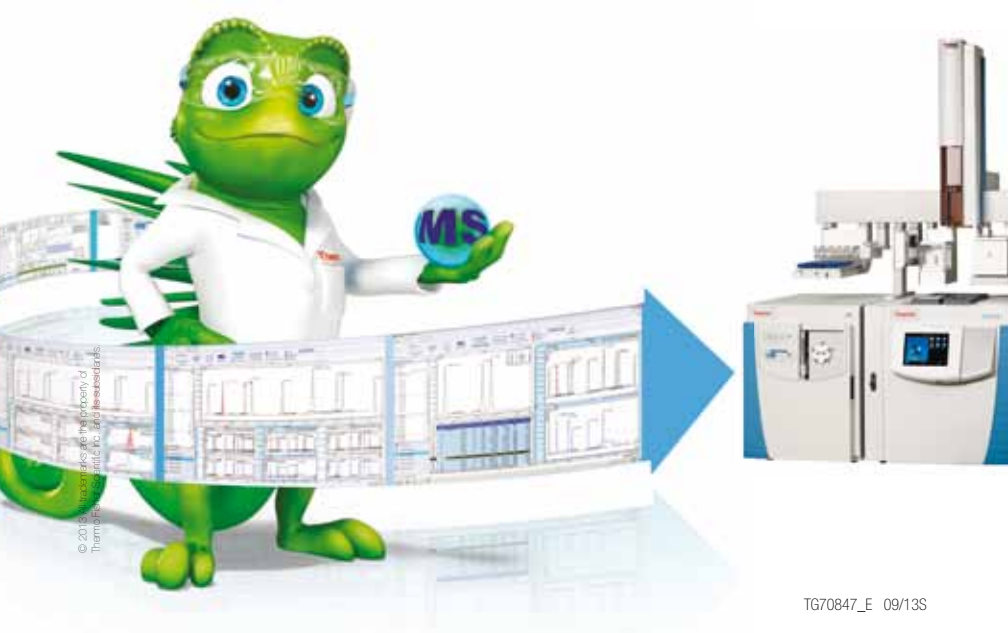

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