

## Watch

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This month's "Column Watch" column is an extensive glossary of definitions and terms used in the liquid-phase separation techniques of high performance liquid chromatography, capillary electrophoresis, and capillary electrochromatography. The glossary should be useful to those just starting to use these separation techniques and can serve as a refresher for long-time users. It provides some of the newer nomenclature recommended by the International Union of Pure and Applied Chemistry.

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Column Watch Editor

# Glossary of Liquid-Phase Separation Terms

The first glossary of common and not-so-common terms and buzzwords for reference to high performance liquid chromatography (HPLC) columns and column technology was published in 1988 (1). It is time for an update because

- many new terms have arisen or, in some cases, their original meanings have expanded or changed;
- the various techniques of capillary electrophoresis (CE) have become well developed and are used in many laboratories throughout the world; and
- the International Union of Pure and Applied Chemistry (IUPAC) published its massive undertaking titled "Nomenclature for Chromatography," which provides guidance and changes in some of the more commonly accepted terms (2).

This month's "Column Watch" will update the earlier glossary and will expand coverage into techniques beyond HPLC. This glossary is not intended to be an in-depth or highly theoretical treatment. For example, we have elected not to cover the myriad terms used in instrumentation, detection, data handling, quantitative analysis, and validation associated with liquid-phase analysis but instead have chosen to use terms that analysts may encounter in everyday laboratory work with columns, phases, and method development. The listing should be helpful to those just starting in HPLC, CE, and related techniques. It also may serve as a refresher for long-time users.

The entire glossary also can be found on the LCGC web site at <http://www.chromatographyonline.com>.

**A**  
 $\alpha$ : See *separation factor*.

**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.

**Activity:** The relative strength of the surface of the packing in adsorption chromatography. For silica gel, the more available the silanol groups, the more active the surface. Activity can be controlled by adding water or other polar modifier that hydrogen bonds to the active sites, thereby reducing the surface activity.

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

**Adjusted retention time ( $t_R'$ ):** A measure of the retention time adjusted for the holdup time;  $t_R' = t_R - t_M$ , where  $t_R$  is the retention time and  $t_M$  is the holdup time (the time it takes for a small, unretained compound that completely permeates the pores to be eluted from the chromatographic column).

**Adjusted retention volume ( $V_R'$ ):** Adjusts the retention volume for the holdup volume;  $V_R' = V_R - V_M$ , where  $V_R$  is the retention volume of the peak of interest and  $V_M$  is the holdup volume (the volume corresponding to the total volume of mobile phase in the column). See also *dead volume* and *holdup volume*.

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

high performance liquid chromatography (HPLC).

**Adsorption:** The process of retention in which the interactions between the solute and the surface of an adsorbent dominate. The forces can be strong forces (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 that 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 LC modes that relies upon adsorption to the surface of an active solid to effect the separation. Silica gel and alumina are the most frequently used normal-phase adsorbents, and molecules are retained by the interaction of their polar function groups with the surface functional groups; for example, silanols of silica. Carbon also is used as an adsorbent in a reversed-phase mode.

**Adsorption isotherm:** 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 in adsorption chromatography. The shape of the adsorption isotherm can determine the chromatographic behavior of the solute; for example, peak tailing, peak fronting, and column 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 size-exclusion chromatography (SEC) are examples of aerogels that can retain their structures even at the high pressures used in HPLC. See also *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 will be eluted unretained. The retained compound later can 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, often in gel-filtration chromatography, with 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. Aluminum oxide is a porous adsorbent that is available with a slightly basic surface; neutral and acidic modifications also can be made. Basic alumina can have advantages over silica, which is considered to have an acidic surface.

**Amino phase:** A propylamino phase used in normal bonded-phase chromatography. It is somewhat reactive for solute molecules such as aldehydes or mobile-phase additives that can react with amines. The amino phase has found some applications as a weak anion exchanger, and it also is used for the separation of carbohydrates with a water-acetonitrile mobile phase. It is a relatively unstable phase.

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

**Analyte:** The compound of interest to be analyzed by injection into and elution from an HPLC column.

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

**Asymmetry:** Factor describing the shape of a chromatographic peak. Chromatographic theory assumes a Gaussian shape and that peaks are symmetrical. A quantita-

tive 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. Other measures of asymmetry are commonly used, especially the *U.S. Pharmacopeia (USP)* method. See Figure 1. See also *Foley-Dorsey equation*.

**Asymmetry factor:** A factor that denotes band shape. The asymmetry factor is 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*) produces a ratio called the peak asymmetry factor (see Figure 1). The ratio is 1 for a symmetrical peak, less than 1 for a fronting peak, and greater than 1 for a tailing peak. The higher the value, the less symmetrical the peak; values greater than 2 are unacceptable.

**Atmosphere (atm):** A measure of the pressure drop across an HPLC column; 1 atm = 14.7 lb/in.<sup>2</sup> (psi). See also *bar* and *pascals*.

## B

**β:** See *phase ratio*.

**B<sub>0</sub>:** See *permeability*.

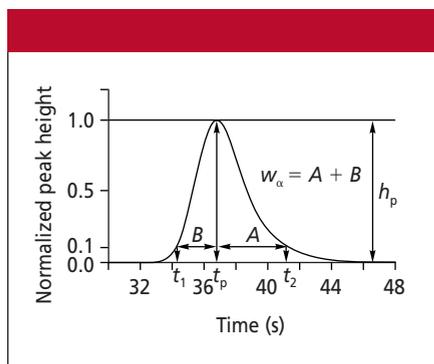
**B solvent:** Usually the stronger solvent in a binary eluent or gradient separation; typically the organic modifier or modifier-rich binary mixture with water in reversed-phase LC.

**B term:** The second term of the van Deemter equation. See also *longitudinal diffusion* and *molecular diffusion term*.

**Backflushing:** A column-switching technique in which a four-way valve placed between the injector and the column allows mobile-phase flow in either direction. Backflushing is used to elute strongly held compounds at the head of a column. It can be used for analyzing these compounds or merely removing them from the column.

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

**Band broadening:** The process of increasing width and concomitant diluting of the chromatographic band as it moves down the column. The peak is injected as a narrow slug and, ideally, each separated component would be eluted as a narrow slug of pure compound if not for the process of band broadening. The measure



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

of band broadening is bandwidth ( $t_w$ ) or, more correctly, the number of theoretical plates ( $N$ ) in the column. Sometimes called band dispersion or band spreading. See Figure 2.

**Bandwidth ( $t_w$ ):** The width of the chromatographic band during elution from the column. It usually is measured at the baseline by drawing tangents to the inflection points on the sides of the Gaussian curve that represents the peak. Small bandwidths usually represent efficient separations; also called peak width. See Figure 2.

**Bar:** A unit of pressure measurement in HPLC equal to 1 atm,  $\sim 15$  lb/in.<sup>2</sup>, or 0.1 MPa.

**BET method:** Developed by Bruner, Emmett, and Teller (BET), a method for measuring surface area that uses nitrogen adsorption–condensation in pores at liquid nitrogen temperature. Pore volume and pore size distribution also can be obtained from BET method calculations.

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

**Binary mobile phase:** Mobile phase comprising two solvents or buffers.

**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.

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

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

**Boxcar chromatography:** See *column switching*.

**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 and the retention factor of the solute. It is useful to determine the total sample capacity of the column for a particular solute.

**Buffer:** A solution that maintains constant pH by resisting changes in pH from

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 pH unit change in 1 L of a buffer solution) or simply the ability of a buffer to withstand injections of a buffered sample solution without changing mobile-phase pH; capacity determined by pH, buffer  $pK_a$ , and buffer concentration.

**C term:** The interphase mass transfer term of the van Deemter equation. See also *mass transfer* and *van Deemter equation*.

**C8:** See *octylsilane*.

**C18:** See *octadecylsilane*.

**C<sub>4</sub>, C<sub>8</sub>, C<sub>18</sub>, etc.:** Refer to the alkyl-chain length of a reversed bonded phase.

**C<sub>5</sub>:** See *Langmuir isotherm*.

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

**Capacity factor ( $k'$ ):** Old term for a chromatographic parameter that measures the degree of retention. Now defined as the *retention factor* ( $k$ ) by the International Union of Pure and Applied Chemistry (IUPAC). See also *retention factor* for method of calculation.

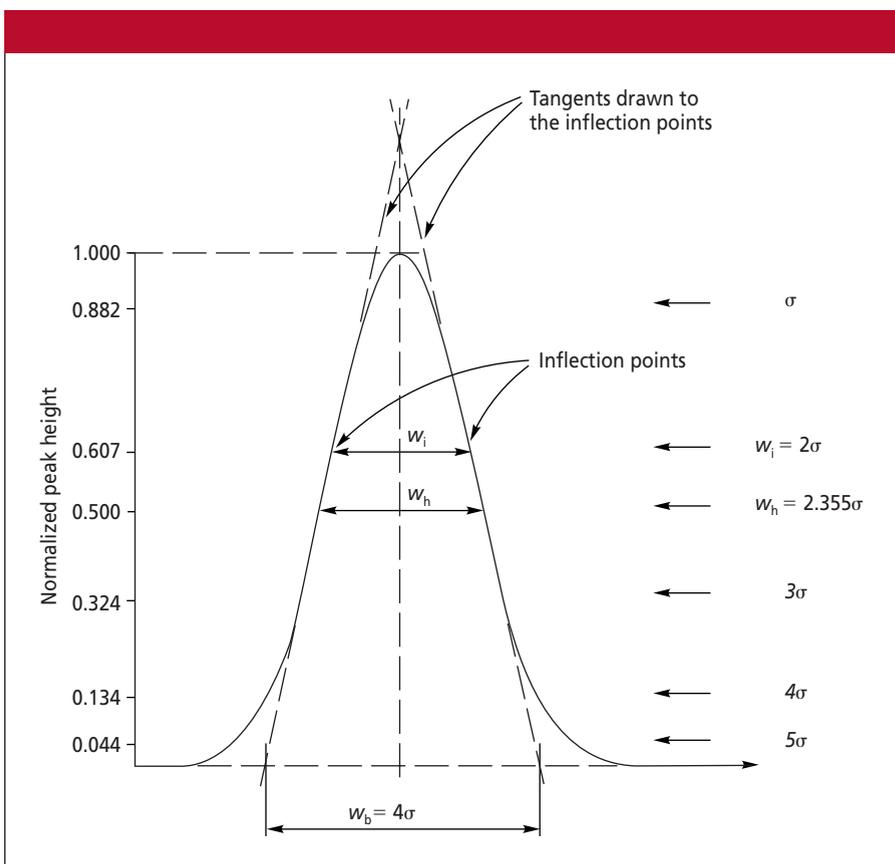
**Capillary column:** Refers to columns with inner diameters less than 0.5 mm.

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

**Capillary gel electrophoresis (CGE):** A technique in which a capillary is filled with, or the walls coated or covalently bonded with, cross-linked polyacrylamide to simulate slab gel electrophoresis; this polymer network uses a sieving mechanism; used for protein, carbohydrate, and DNA separations such as fingerprinting and sequencing.

**Capillary isoelectric focusing:** Separation is based on isoelectric points of proteins; the capillary is filled with solution; the sample is introduced into the capillary in the presence of ampholytes; under the application of an electric field, the protein migrates until it reaches a pH at which it is neutralized and maintains that position in the capillary.

**Capillary LC:** Generally refers to HPLC performed in a fused-silica or other type of



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

capillary column; the inner diameters typically are less than 0.5 mm; has also been called micro-LC.

**Capillary micellar electrochromatography:** The CEC version of micellar electrokinetic capillary chromatography (MEKC).

**Capillary tubing:** Tubing to connect various parts of a chromatograph and direct flow to the proper places. Most capillary tubing used in HPLC is less than 0.020 in. in inner diameter. The smallest useful inner diameter is approximately 0.004 in.

**Capillary zone electrophoresis (CZE):** CE performed in an open fused-silica capillary tube with and without various additives and capillary coatings; also called *open-tube capillary zone electrophoresis*.

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

**Carrier:** A term most often used in affinity chromatography; refers to the support that binds the active ligand, usually by a covalent bond; can also refer to the support in other chromatography modes such as liquid-liquid chromatography.

**Carrier gas:** The mobile phase in gas chromatography (GC).

**Cartridge column:** A column type that has no endfittings and is held in a cartridge holder. The column comprises a tube and packing 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.

**CE:** Capillary electrophoresis.

**CEC:** See *capillary electrochromatography*.

**CGE:** See *capillary gel electrophoresis*.

**CZE:** See *capillary zone electrophoresis*.

**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 ( $C_8$ ,  $C_{18}$ , etc.). It specifically excludes the short chains — typically methyl, isopropyl, and *sec*-butyl groups — that also are 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, which in turn allows band broadening to

occur. The voids are created by poor packing or erosion of the packed bed.

**Chemisorption:** Sorption caused by a chemical reaction with the packing. Most of these interactions are irreversible and usually occur 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.

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

**Chiral stationary phases:** Stationary phases that are designed to separate enantiomeric mixtures. The phases can be coated or bonded to solid supports, created in situ on the surface of the solid support, or exist as 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 (octadecyl, octyl, etc.) will dictate the hydrophobicity of the resulting bonded phase; alkoxysilanes can be used but are less reactive.

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

**Chromatograph:** As a noun: a device used to implement a chromatographic separation. As a verb (IUPAC): the act of separating by elution through a chromatographic bed.

**Classification:** The process of sizing column packing particles; generally in HPLC, 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 centrifugal air techniques.

**Column back pressure:** See *head pressure*.

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

**Column length (L):** The length of chromatography column in HPLC or capillary in CE used to perform the liquid-phase separation.

**Column performance (N):** Refers to the efficiency of a column; the number of theoretical plates for a given test compound.

**Column plate number (N):** Denotes the column efficiency; the larger the plate number, the more theoretical plates the column possesses; a typical well-packed column with a 5- $\mu\text{m}$   $d_p$  porous packing in a 15 cm  $\times$  4.6 mm column should provide 10,000–12,000 plates.

**Column switching:** Using multiple columns connected by switching valves for better chromatographic separations or 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 detectors; sometimes called *multidimensional chromatography*.

**Column volume (V<sub>c</sub>):** The volume of the unpacked column;  $V_c = A_c L$ , where  $A_c$  and  $L$  are the cross-sectional area of the tube and the tube length, respectively.

**Competing base:** Adding a small basic compound such as triethylamine or dimethyloctylamine at 10–50 mM concentration to the mobile phase in reversed-phase chromatography to inhibit basic analytes from interacting with residual silanols; works by the law of mass action because concentration of competing base is much greater than analyte. See also *additive*.

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

**Controlled surface porosity support:** Same as *porous-layer bead* and *pellicular packing*.

**Counterion:** The ion in solution used to displace the ion of interest from the ionic site in an ion-exchange process. 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 by a selector valve. Fractions from the first column can be selectively transferred to the second and third columns for additional separations. This term also is 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 usually is described in micromoles per

square meter or in terms of percentage carbon (w/w).

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

**Cross-linking:** During the process of copolymerization of resins to form a three-dimensional matrix, a difunctional monomer is added to form cross-linkages between adjacent polymer chains. The degree of cross-linking is determined by the amount of the 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.

**Cyclodextrins:** Cyclic oligomers of several D-(+)-glucopyranose units used in chiral HPLC and CE separations; popular ones are named  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrins; they have a truncated cone shape, a relatively hydrophobic cavity, and primary and secondary hydroxyl groups at their ends; they separate on the basis of differential inclusion of enantiomers; modified cyclodextrins with derivatized hydroxyl groups also are used for selectivity modification.

**D** **Dead volume ( $V_M$ ):** The *column* dead volume comprises the entire space accessible to a small molecule that can fully permeate all pores of a packing material. It includes the interstitial volume and the unoccupied pore volume. It is denoted as  $V_M$ . The *system* dead volume includes the additional volume in the tubing that connects the injector and detector to the column. The system dead volume usually is approximated by injecting a small, essentially unretained species. Uracil, acetone and thiourea are most commonly used species in reversed-phase chromatography. See also *adjusted retention volume*, *holdup volume*, and *void volume*.

**DEAE:** See *diethylaminoethyl*.

**Degassing:** The process of removing dissolved gas from the mobile phase before or during use. Dissolved gas may come out of solution in the detector cell and cause baseline spikes and noise. Dissolved air can affect detectors such as electrochemical (by reaction) or fluorescence (by quenching) detectors. Dissolved gases also can cause

pumps to lose their prime. Degassing is performed by heating the solvent, helium sparging, or using vacuum (in a vacuum flask) or on-line evacuation from a tube made of a gas-permeable substance such as polytetrafluoroethylene (PTFE).

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

**Desalting:** Technique in which low molecular weight salts and other compounds can be removed from nonionic and high molecular weight compounds. An example is using a reversed-phase packing to retain sample compounds by hydrophobic effects yet allowing salts to pass through unretained. Using an SEC column to exclude large molecules and retain lower molecular weight salts is another example.

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

**Diethylaminoethyl (DEAE):** A popular weak anion-exchange functionality (typically attached to cellulose or Sepharose [Amersham Pharmacia Biotech]) used for separating biomolecules.

**Diffusion coefficient ( $D_M$  or  $D_S$ ):** A fundamental parameter of a molecule in gas, solution ( $D_M$ ), or the stationary phase ( $D_S$ ). Expressed in square centimeters per second.  $D_M$  is dependent on the molecular weight of the solute, temperature, solvent viscosity, and molar volume of the solute. A typical value for a 100-Da molecule in reversed-phase chromatography at room temperature is  $10^{-5}$  cm<sup>2</sup>/s.

**Diol phase:** A hydrophilic phase that is useful in normal and reversed phase. It is a diol structure (two -OH groups on adjacent carbon atoms in an aliphatic chain). In normal-phase work, it is less polar than silica. It has been used to separate proteins and polypeptides in reversed-phase chromatography.

**Displacement chromatography:** A chromatographic process in which the sample is placed onto the column head and then is displaced by a compound that is more strongly sorbed than the compounds of the original mixture. Sample molecules then are 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-scale HPLC applications.

**Distribution constant (coefficient) ( $K_C$ ):** The total equilibrium concentration of a

component in all forms or on the stationary phase divided by the total equilibrium concentration of the component in the mobile phase; also called the distribution coefficient or the partition coefficient in partition chromatography. In partition chromatography,  $K_C$  is used when the concentration in the stationary phase is expressed per unit volume of the phase ( $V_R = V_M + K_C V_S$ ). In a solid stationary phase,  $K_C$  is used and is expressed per mass (weight) of the dry solid phase. In adsorption chromatography with a well-characterized adsorbent of known surface area, the concentration in the stationary phase is expressed per unit surface area.

**$D_M$ :** See *diffusion coefficient*.

**$d_p$ :** See *particle size*.

**$D_S$ :** See *diffusion coefficient*.

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

**Dwell volume:** 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 an LC column. Important in gradient elution or in isocratic elution situations when changes in solvent composition are made 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 adding a substance to the mobile phase that adsorbs onto (or absorbs into) 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 less than pH 3 to protonate silanols and negate their effect. Another example is coating the phase with a hydrophilic polymeric material to prevent adsorption of proteins.

**E**  **$E$ :** See *separation impedance*.

**$\epsilon$ :** See *interparticle porosity*.

**Eddy dispersion (diffusion) term ( $\lambda$ ):** The A term in the van Deemter equation. It is the contribution to plate height from the heterogeneity in axial velocities as a result of the particle size and geometry of the packing, as well as wall effects;  $A =$

$2\lambda d_p$ , where  $\lambda$  is an empirical column constant. Typical values of  $\lambda$  for well-packed columns are 0.8–1.0. Some theories of chromatography indicate a velocity-dependent contribution to the height equivalent to a theoretical plate (HETP) from this process. Also known as eddy diffusion, flow-heterogeneity induced broadening, and the multipath term. See also *van Deemter equation*.

$\epsilon_e$ : See *interstitial porosity*.

**Effective capillary length:** The distance between the point of sample addition and the point of detection in CE. For on-capillary detection in which the column is used as the flow cell in UV detection, this length is shorter than the capillary length.

**Effective plate height ( $H_{\text{eff}}$ ):** The column length divided by the effective plate number.

**Effective theoretical plates ( $N_{\text{eff}}$ ):** Also called the effective plate number by IUPAC. The true number of plates in a column, because it corrects theoretical plates for dead volume.  $N_{\text{eff}} = 16[(t_R'/w_b)^2]$ , where  $t_R'$  is the adjusted retention time and  $w_b$  is the bandwidth of the peak (see Figure 2). It is a better figure of merit than simple plate number for comparing devices of very different geometries and phase ratios.

**Efficiency ( $N$  or  $H$ ):** A measure typically determined by the number of theoretical plates ( $N$ ) calculated from the equation  $N = 16(V_R/w_b)^2 = 16(t_R/w_b)^2$ , where  $w_b$  is the peak width measured at the base (see Figure 2). If the peak width is measured at half height, the following equation is used:  $N = 5.545(V_R/w_b)^2$ . The plate height ( $H$ ) or HETP is determined by  $H = L/N$ . The efficiency of asymmetric peaks is better determined from the peak centroid and variance by mathematical analysis of the peak shape. See also *Foley–Dorsey equation*.

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

$\epsilon_i$ : See *intraparticle porosity*.

**Electroosmotic flow:** See *electroosmotic flow*.

**Electromigration injection:** Inlet end of CE capillary is placed in sample solution and voltage is applied for a set time; analytes move from sample vial into capillary; discrimination effects may occur because compounds of differing charges will migrate at different rates.

**Electroosmotic flow ( $v_{eo}$ ):** Bulk flow of solvent within capillary caused by presence of zeta potential (electric charge) at the capillary walls and absence of flow resis-

tance. Most likely source of zeta potential is presence of ionized silanols at the fused-silica surface or intentional coating of the capillary wall with an ionic phase. Depending upon zeta potential, electroosmotic flow may be toward anode or cathode and contributes to overall retention in CE techniques.

**Electrophoresis:** The movement of sample ions under the influence of an applied voltage.

**Electrophoretic mobility ( $\mu$ ):** Characteristic of a given ion in a given medium and at a given temperature in CE analyses; proportional to the charge of ion and inversely proportional to solution viscosity and the ion's radius.

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

**Eluent:** The mobile phase used to perform a separation.

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

**Eluotropic series:** A series of solvents (eluent) 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 the low end of the scale, an intermediate solvent such as methylene chloride 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 reversed-phase chromatography, the reverse order of strength would be observed; water would be weak and acetonitrile strong. Thus, when developing a method or running a gradient, an eluotropic series is useful for selecting solvents. See also *Snyder  $e^o$* .

**Elute:** To chromatograph by elution chromatography. The term *elute* is preferred over *develop*, which was used in older nomenclature.

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

**Elution chromatography:** The most commonly used chromatographic method in which a sample is applied to the head of the column as a narrow zone and individual analytes are separated and eluted from the end of the column. Compare with *displacement chromatography* and *frontal analysis*.

**Elution volume ( $V_R$ ):** Refers to the volume of mobile phase necessary to elute a solute from a column. It is the volume from the point of injection to the volume at maximum concentration (apex) for a

symmetrical peak;  $V_R = Ft_R$ , 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 most often is used for the separation of ion-exchange resins that require 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 upon their density and particle size. They are removed at certain levels in the tube. High-purity spherical silica gels sometimes are sized by elutriation.

**Endcapping:** A technique used to remove silica gel silanol groups 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 (such as trimethylchlorosilane or 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. Endcapping reactions also are used to remove terminal silanol groups from polymeric phases.

**Endfitting:** The fitting at the end of the column that permits connection to the injector or detector. Most HPLC endfittings have frits to contain the packing and low dead volumes for minimum band spreading. They usually are constructed of stainless steel, but polyetherether ketone (PEEK) and other polymeric materials also are used.

**Enzymophoresis:** A tandem format in which a short fused-silica capillary containing immobilized enzyme on the inner wall is coupled with a CZE capillary; an enzymatic reaction occurs with the injected sample, and the products and unreacted substances enter the separation capillary; used to improve separations, detection, or analyte preconcentration.

$\epsilon_T$ : See *total porosity*.

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

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

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

**Exclusion limit:** 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 known by their exclusion limit. For example, a  $10^5$  column of porous silica gel will exclude any compounds with a molecular weight greater than 100,000, based on a polystyrene calibration standard.

**Exclusion volume ( $V_0$ ,  $V_{ei}$ ):** The minimum retention volume of a molecule on an SEC packing in which all molecules larger than the size of the largest pore are totally excluded. These molecules are incapable of penetrating the pores and are eluted at the interstitial (interparticle) volume of the column.

**Exponentially modified Gaussian peak:** An asymmetric peak resulting from passing a Gaussian peak through a detector that is excessively slow or has an excessive volume. Frequently used to model peak tailing arising from the column per se. The basis for the Foley–Dorsey equations. See also *Foley–Dorsey equation*.

**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 a column. Sources of band broadening can include the injector design, 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 comprises the volumes of the injector, connecting lines and frits, and the detector. It determines the *extracolumn effects*.

## F

**F:** See *flow rate*.

$\Phi$ : See *flow resistance parameter*.

**Fast LC:** Use of HPLC of short columns (1.5–7 cm) with conventional inner diameters (2–6 mm) packed with small particles (3- or 5- $\mu\text{m}$   $d_p$ ). Separation times in the range of minutes, or even seconds, are common.

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

**Flash chromatography:** A very fast form of classic LC used by synthetic organic chemists for rapid purification. Performed

primarily in the normal-phase mode, sometimes with reversed-phase chromatography.

**Flow rate (F):** The volumetric rate of flow of a mobile phase through an LC column. Typical flow rates are 1–2 mL/min for a conventional 4.6-mm i.d. HPLC column.

**Flow resistance parameter ( $\Phi$ ):**  $\Phi = d_p^2/B_0$ , where  $B_0$  is permeability. See also *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 these phases have different selectivities than hydrocarbon phases.

**Foley–Dorsey equation:** A correction of the plate count and retention time for peak tailing from extracolumn sources of broadening. See reference 3.

**FPLC:** See *fast protein LC*.

**Fractionation range:** Refers to the operating range of a gel or packing in SEC. This range is where a 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 and are eluted (unseparated) at the permeation volume.

**Free solution CE:** See *capillary zone electrophoresis*.

**Frit:** The porous element at either end of a column that contains the column packing. It is placed at the very ends of the column tube or, more commonly, in the endfittings. Frits can be stainless steel or other inert metal or plastic such as porous PTFE or polypropylene. The frit porosity must be less than the smallest particle in the HPLC column; otherwise particles will pass through the frit, and the 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 the first-eluted compound, the third-least-sorbed compound with the first and second compound and so on until the original sample is eluted at the column exit. Frontal analysis is seldom used and is mainly a preparative technique.

**Frontal chromatography:** 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. The peak has an asymmetric distribution with a leading edge. The asymmetry factor for a fronting peak has a value of less than one. Tailing is the opposite effect. Fronting can result at high sample loads because of positive curvature in the isotherm and from using poorly packed columns.

## G

$\gamma$ : The obstruction or tortuosity factor. Molecular diffusing term. See also *tortuosity*.

**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. Using the peak maximum position as a measure of retention and the efficiency equations mentioned above assume Gaussian peak shape. See Figure 2.

**Gaussian peak:** A peak whose shape conforms closely to the equation:  $C = C_{\text{max}} \exp[-(t - t_R)^2/2\sigma^2]$ .

**Gel:** The solid packing used in gel chromatography or gel-permeation chromatography (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. Performed with aqueous mobile phases. Generally refers to molecular size separation performed on soft gels such as polydextrans, but analysts also can use highly cross-linked polymers, silica gels, and other porous media. Most gel-filtration separations involve biopolymers and water-soluble polymers such as polyacrylic acid.

**Gel-permeation chromatography (GPC):** SEC performed with organic mobile phases used for the separation and characterization of polymers. SEC with aqueous mobile phases is called aqueous GPC, GFC, or aqueous SEC.

**GFC:** See *gel-filtration chromatography*.

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

**GPC:** See *gel-permeation chromatography*.

**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 in which solvents are blended to achieve the proper strength.

**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 packing:** A reversed-phase packing material consisting 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.

**Guard column:** A small column placed between the injector and the analytical column. It protects the analytical column from contamination by sample particulates and strongly retained species. The guard column usually is packed with the same material as that in the analytical column and is often of the same inner diameter. It is much shorter, costs less, and usually is discarded when it becomes contaminated. Integrated guard-analytical column systems often are preferred to minimize extra-column effects caused by connecting tubing with separate guard and analytical columns.

## H

**h:** Reduced plate height. Defined as  $HETP/d_p$ , where HETP is the height equivalent to a theoretical plate and  $d_p$  is the particle diameter. See also **reduced plate height**.

**H:** Same as **HETP**. See also **efficiency**.

**η:** See **viscosity**.

**Head pressure ( $\Delta p$ ):** The difference in pressure between the inlet and outlet of a column measured in pounds per square inch. Governed by the following approximate equation for a column packed with spherical particles of typical internal porosity (0.5):  $\Delta p = 3000L\eta/t_M d_p^2$ , where  $L$  is the column length in centimeters,  $\eta$  is the mobile-phase viscosity in centipoise,  $t_M$  the column holdup time in minutes, and  $d_p$  is the particle diameter in micrometers. Pressure can be expressed in pounds per square inch, bars, atmospheres, or pascals.

**Heart cutting:** Refers to collection of the center of the peak at which purity should

be maximum in preparative LC. The term also is used in column switching.

**H<sub>eff</sub>:** See **effective plate height**.

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

**HETP:** Height equivalent to a theoretical plate. A carryover from distillation theory; a measure of column efficiency;  $HETP = L/N$ , where  $L$  is column length and  $N$  is the number of theoretical plates. HETP should be approximately  $2-3 d_p$  for 5- $\mu$ m particles with a typical well-packed HPLC column, HETP (or  $H$ ) values usually are in the range of 0.01–0.03 mm. See also **efficiency** and **h**.

**High performance CE:** A technique in which small-diameter capillaries, buffered conducting solutions, and high voltages (as much as 30,000 V) separate ionic molecules based on their differential electrophoretic mobilities. Nonionic (neutral) molecules can be separated by MEKC.

**High performance liquid chromatography (HPLC):** The modern, fully instrumental form of liquid-phase chromatography technique that uses small particles and high pressures. Sometimes called high-pressure LC.

**Holdup volume ( $V_M$ ):** The total volume of mobile phase in the column regardless of where it exists;  $V_M = V_c + V_i$ , where  $V_c$  is the interstitial volume and  $V_i$  is the intraparticle volume. Also called the column void volume. IUPAC indicates that use of the term **dead volume** should be eliminated for this concept. The use of dead volume is limited to regions not swept by the flowing mobile phase system. Holdup volume is measured by injecting an unretained species that fits in all the pores. See also **interstitial porosity** and **intraparticle porosity**.

**HPLC:** See **high performance liquid chromatography**.

**Hybrid silica:** Silica gel comprising both organic and inorganic moieties with hybrid properties of polymeric packings and silica packings. Synthesized from silanes containing organic functionality. Different selectivity but better high-pH stability than bare or uncoated silica gel.

**Hydrodynamic injection:** Used in CE. See also **hydrostatic injection**.

**Hydrodynamic volume:** The molecular volume defined by the effective diameter of a molecule in free solution at which the hydrodynamic sphere would be a sphere defined by the molecule as it revolves around its central axis in solution. Term

used in SEC to define molecular shape and to explain why molecules with the same molecular weight often have different elution volumes. Measured by determining the Stokes radius.

**Hydrophilic:** Greek word for water loving. Refers to stationary phases that are fully compatible with water and to water-soluble molecules in general. Many columns used to separate proteins — such as ion-exchange, SEC, and affinity columns — are hydrophilic in nature and should not irreversibly sorb or denature protein in an aqueous environment.

**Hydrophilic interaction chromatography:** Using ion-exchange columns to separate compounds on the basis of nonionic interactions. Columns are used to separate hydrophilic peptides with a gradient from organic to aqueous solvents. Solutes are separated based on their hydrophilicity rather than hydrophobicity.

**Hydrophobic:** Greek word for water fearing. Refers to stationary phases that are incompatible with water or to molecules that in general 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:** A technique in which weakly polar (nonhydrocarbonaceous) packings are used to separate molecules by the interactions of their hydrophobic moieties and the hydrophobic sites on their packing surface. High concentrations of salt solutions are used in the mobile phases, and separations are generated by changing the salt concentration. The technique is analogous to salting-out molecules from solution. Gradients are run by decreasing the salt concentration. The technique often is used to separate proteins that are sensitive to denaturation by the organic solvents used in regular reversed-phase chromatography. Usually little or no organic solvent is used in the mobile phase in hydrophobic interaction chromatography.

**Hydrostatic injection:** Also called hydrodynamic injection. Using gravity (differential pressure) to make an injection into a CE capillary. Vial containing sample solution is raised at a set distance above the ground end of the column and column inlet end is placed into vial for set time. After determining flow rate, users can determine injected volume. Good for wide-bore capillaries.

**Hydroxyapatite:** A porous calcium

hydroxy phosphate solid that chemically resembles bone and tooth. Used as a packing material in biochromatography for nucleic acid constituents, monoclonal antibodies, and proteins.

**Hyphenated techniques:** Refers to the family of techniques best known by their acronyms, including LC–mass spectrometry (MS), LC–Fourier transform IR spectroscopy (FTIR), and LC–MS–MS. See also *multidimensional chromatography*.

**IC:** See *ion chromatography*.

**Immobilized metal-affinity chromatography:** See *metal-affinity chromatography*.

**Imprinted phases:** Polymer and silica phases generated in the presence of a template or printing molecule. These phases have enhanced selectivity for the templating molecule.

**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 also *size-exclusion chromatography*.

**Indirect detection:** Used for non-UV absorbing or nonfluorescing analytes. A UV-absorbing or fluorescent compound added to the mobile phase 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, it produces a positive peak. When a negative signal is detected, the detector signals are reversed to the output device.

**Infinite diameter column effect:** 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. Phenomenon observed by John Knox, who 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.

**Inlet:** The initial part of the column where the solvent and sample enter. An inlet frit usually holds the packing in place and, in some cases, protects the packed bed.

**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 prevents sample particles from entering the packed bed or column inlet frit.

**Interparticle porosity ( $\epsilon_e$ ):** The interparticle volume of a packed column per unit column volume;  $\epsilon_e = V_e/V_c$ , where  $V_e$  is the interstitial volume and  $V_c$  is the total column volume. See also *interstitial porosity*.

**Interparticle volume ( $V_o$ ):** The volume of mobile phase located outside the particles.

**Interstitial porosity ( $\epsilon_e$ ):** The fraction of the volume in the column located in the interparticle (interstitial) space;  $\epsilon_e = V_e/V_c$ .

**Interstitial velocity ( $u_e$ ):** The actual velocity of the eluent as it moves through the column flowing around the particles;  $u_e = F/A_c\epsilon_e$ . The interstitial velocity is the basis for computing the reduced velocity.

**Interstitial volume ( $V_o$ ):** The volume between the particles. It does not include the volume in the pores of the particles. Also called the excluded volume (see SEC) and interparticle volume. Measured by injecting a molecule that 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 ( $\epsilon_i$ ):** The fraction of the particle volume that is the pore volume;  $\epsilon_i = V_{\text{pore}}/V_{\text{particle}}$ .

**Intraparticle volume ( $V_i$ ):** The volume inside the pores of the particles. Also called the internal and included volume. Can be measured by the BET method 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 often are used. IC is practiced in two forms: In suppressed IC, a second column or a membrane separator is used to remove the buffer counter ion from the analyte and simultaneously replace it with a hydrogen or hydroxide ion that concomitantly converts the buffer to an uncharged species thereby suppressing background and enhancing sensitivity. In nonsuppressed IC, low-concentration, weakly conducting buffers are carefully selected, the entire effluent is passed through the detector, and ions are detected above the background signal.

**Ion-exchange capacity:** The number of

ionic sites on the packing that can participate in the exchange process. The exchange capacity is expressed in milliequivalents per gram. A typical styrene–divinylbenzene strong anion-exchange resin may have 3–5 mequiv/g 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, usually with 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 other solution parameters such as pH, ionic strength, and counterion type. Ion chromatography basically is an ion-exchange technique.

**Ion exclusion:** The process in which ionized solutes can be separated from un-ionized or partially ionized solutes using ion-exchange resins. Separation results from Donnan potential in which 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 nonionic solutes. Ion exclusion occurs in reversed-phase chromatography when anions are separated at pH values at which the silanol groups are ionized.

**Ion-moderated partitioning chromatography:** A technique used for separating 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-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 usually are ionic compounds that contain a hydrocarbon chain, which 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 also can 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 chromatography. Also known as ion-interaction chromatog-

raphy or dynamic ion-exchange chromatography, which stresses that users sometimes do not know the precise mechanistic details of how the additive controls retention.

**Ion retardation:** Refers to using amphoteric ion-exchange resins, which 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  $pK_a$  value. Useful for improving peak shape of weak acids and bases in reversed-phase chromatography.

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

**Irreversible adsorption:** When a compound with 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. See also *chemisorption*.

**Isocratic:** Using a time invariant–eluent composition in LC.

**Isotachopheresis:** Ionic species are separated in CE according to differences in mobilities by applying an electric field. Isotachopheresis is performed with a discontinuous buffer in which the sample zone is located between the background electrolyte of higher (leading electrolyte) and lower (terminal electrolyte) electrophoretic mobilities.

**Isotachopheretic focusing:** Using the principles of isotachopheresis to focus or concentrate analytes at the head of the column to provide concentration sensitivity enhancement.

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

**Isothermal chromatography:** Using conditions of constant temperature. The vast preponderance of all LC is performed under isothermal conditions.

vent within a capillary caused by voltage applied to the column and the resulting current. The temperature is related to electric field strength, conductivity of the solution and the capillary radius. Minimizing heat generation and maximizing heat dissipation is critical in CZE because thermal gradient and other heat-related effects can adversely affect CE's high efficiency.

**K:** See *retention factor*.

**$k'$ :** An old term that has been replaced by the IUPAC-approved term, retention factor ( $k$ ).

**$K$ :** See *partition coefficient*.

**$k_{A/B}$ :** See *selectivity coefficient*.

**$K_C$ :** See *distribution constant (coefficient)*.

**Kieselguhr:** A diatomaceous earth used in column chromatography and also as a sample cleanup media. Only weakly adsorptive, it can be used as a support in liquid–liquid chromatography. Rarely used in HPLC.

**Knox equation:** A modification of the van Deemter equation developed by John Knox in which the A term that represents eddy dispersion multiplied by  $u^{1/2}$ , where  $u$  is the interstitial eluent velocity. Usually written in terms of the dimensionless or reduced plate height ( $h$ ) and reduced velocity ( $v$ ) as  $h = Av^{1/2} + B/v + Cv$ . See also *van Deemter equation*.

**L:** See *column length*.

**Laminar flow:** The smooth time-invariant flow that develops when a liquid is moving under conditions in which viscous forces dominate inertial forces. Laminar flow is characterized by a low Reynolds number (see *Reynolds number*). In a cylindrical tube, fluid streams in the center flow faster than those at the tube wall, which results in a radially parabolic distribution in axial fluid velocity. This nonuniformity of axial velocities in the interstices in a packed bed also causes substantial peak broadening in packed columns.

**Langmuir 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.

**LC:** See *liquid chromatography*.

**Leading electrolyte:** In isotachopheresis, the electrolyte that contains the ion with the highest mobility above that of any

sample component ions for strong electrolytes.

**Ligand:** In ligand-exchange chromatography, it refers to the analyte that undergoes ligand exchange with the stationary phase. In affinity chromatography, it refers to the biospecific material — enzyme, antigen, or hormone — coupled with the support (carrier) to form the affinity column. In bonded-phase chromatography, it refers to 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. For example, copper salt can be added to the mobile phase for the chelation and separation of amino acids. Chelating resins function in a similar manner: chelating groups are chemically bonded to the polystyrene backbone.

**Linear elution adsorption chromatography:** Refers to adsorption chromatography performed in the linear portion of an adsorption isotherm. A term coined by Lloyd Snyder.

**Linear velocity ( $u$ ):** The velocity of the mobile phase moving through the column. Expressed in centimeters per second. Related to flow rate by the cross-sectional area of the column. Determined by dividing the column length ( $L$ ) by the retention time of an unretained compound. See also *void time*.

**Liquid chromatography (LC):** A separation technique in which the mobile phase is a liquid. Most often performed in a column.

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

**Liquid–solid chromatography:** Same as *adsorption chromatography*.

**Loading (phase loading versus sample loading):** The amount of stationary phase coated or bonded onto a solid support. In liquid–liquid chromatography, the amount of liquid phase in milligrams of per gram of packing. In bonded-phase chromatography, the loading may be expressed in micromoles per square meter or percentage carbon (w/w). Also called coverage or surface coverage. An alternate and unrelated meaning is the amount of sample mass injected on an analytical- or preparative-scale column; preparative-scale columns often are operated in an overloaded condi-

**Joule heating:** Electrical heating of sol-

tion for throughput reasons.

**log  $k_w$ :** The extrapolated intercept of a plot of log  $k$  versus volume fraction of organic modifier in reversed-phase LC. See also **S**.

**Longitudinal diffusion:** Same as *molecular diffusion term*. B term in van Deemter equation. See also *van Deemter equation*.

**M**    
 $\mu$ : See *electrophoretic mobility*.

**Macroporous resin (macroreticular):** Cross-linked ion-exchange resins that have molecular-scale micropores and also macropores of several hundred angstroms. These highly porous resins have large internal surface areas that are accessible to large molecules.

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

**Mean pore diameter:** The average diameter of the pore of a porous packing. It most commonly is determined by the BET method and is reported as fourfold the specific pore volume divided by the specific surface area ( $4V/A$ ) 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 many paths can allow a molecule to access any part of the pore space. In SEC, the packings have different pore diameters; 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. Pore diameters greater than 300 Å are used for the separation of biomolecules. Pores usually are classified as micro (<20 Å), meso (20–500 Å), and macro (>500 Å).

**MECC:** See *micellar electrokinetic capillary chromatography*.

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

**MEKC:** See *micellar electrokinetic capillary chromatography*.

**Metal-affinity chromatography:** A special form of ligand-exchange chromatography used to separate 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 or multifunctional carboxylate or phenol.

**Method development:** A process for optimizing the separation, including the sample pretreatment, 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, if not optimum, separation.

**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:** Adding micelles to the mobile phase to cause separation. The micelles may act as displacing or partitioning agents and provide another parameter to change selectivity. Surfactants at concentrations greater than their critical micelle concentration are used in micellar chromatography and in MEKC.

**Micellar electrokinetic capillary chromatography (MEKC, MECC):** Similar to micellar chromatography. Used for the CE separation of neutral compounds under electroosmotic flow conditions. Detergent or surfactant is added to the running buffer at a concentration to make it greater than its critical micelle concentration. Analytes partition by hydrophobic interactions into and out of the micelles while they are moving through the capillary under the influence of electroosmotic flow; the result is that neutral compounds are separated by their differential migration down the capillary.

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

**Microbore:** Refers to the use of smaller-than-usual inner diameter columns in

HPLC. Columns of 2 mm and less are considered to be microbore sizes. Inner diameters of 0.5 mm and smaller are considered micro-LC columns.

**Microchip devices:** Microdevices based on silicon, glass, and other types of microfabricated chips in which experiments can be miniaturized into single- or multichannel microfluidic circuits. These devices can be used for CE and CEC. They should be low cost and disposable. Using microdevices for separation currently is in its infancy, and applications should expand with time.

**Microparticulate:** Refers to the small particles used in HPLC. Generally packings with a particle diameter of less than 10  $\mu\text{m}$  that are totally porous are considered microparticles.

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

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

**Migration rate:** See *electrophoretic mobility*.

**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. Distinct from holdup time ( $t_M$ ).

**Minimum plate height:** The minimum of the van Deemter curve that results from a plot of  $H$  versus  $v$ . This value represents the most theoretical plates that can be obtained for a certain column and mobile-phase system. Usually occurs at excessively low flow rates. Also known as the optimum plate height. It typically is two- to threefold the particle diameter of well-packed columns.

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

**Mixed-mode separation:** A separation that occurs in a single column caused by the retention and selectivity provided by a dual-retention mechanism. For example, a reversed-phase column with residual

silanols at intermediate-to-high pH values can separate by hydrophobic interaction and ionic interaction by the ionized silanols. Sometimes mixed-mode separations can be quite beneficial to the selectivity (band spacing), but they can cause peak asymmetry, and the precise balance of interactions may be difficult to reproduce with subsequent packing batches.

**Mobile phase:** The solvent that moves the solute 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.

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

**Mobile-phase velocity ( $u_M$ ):** The velocity at which the mobile phase percolates through the bed of particles;  $u_M = L/t_M$ , where  $L$  is column length and  $t_M$  is holdup time. See also *adjusted retention volume*, *holdup volume*, and *dead volume*.

**Mobility:** See *electrophoretic mobility*.

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

**Molecular diffusion term (B term):**

Refers to the B term (second term) of the van Deemter equation. Also called longitudinal or axial diffusion term. It dominates band broadening only at very low flow rates below the minimum plate height at which the diffusion of individual solutes can occur in a longitudinal (lengthwise) direction on the column. The contribution to the B term arises from diffusion in the mobile phase and is  $2\gamma D_M$ , where  $\gamma$  is the obstruction factor (typically 0.6–0.8) and  $D_M$  is the diffusion coefficient. See also *van Deemter equation*.

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

**Molecularly imprinted phases:** See *imprinted phases*.

**Monodisperse particles:** Particles that fall into a narrow range of diameters. See also *polydisperse particles*.

**Monomeric phase:** Refers to a bonded phase in which single molecules are

bonded to a support. For silica gel, monomeric phases are prepared by the reaction of an alkyl- or aryl- monochloro- or alkoxy silane. Polymeric phases generally are prepared from a di- or trichlorosilane or an alkoxy silane reactant in the presence of water.

**Moving zone:** To be distinguished from the mobile phase, this zone is the fraction of the mobile phase in the column that occupies the interstitial spaces. See also *stationary phase*.

**Multidimensional chromatography:**

The use of two or more columns or chromatographic techniques to generate a better separation. It is useful for sample cleanup, increased resolution, increased throughput, and increased peak capacity. It can be used off-line by collecting fractions and reinjecting them onto a second column or on-line by using a switching valve. Also called coupled column chromatography, column switching, multicolumn chromatography, and boxcar chromatography.

**N** See *peak capacity*.

**N:** The number of theoretical plates;  $N = 16(t_R/w_b)^2$ , where  $t_R$  is retention time and  $w_b$  is the base width of the peak. A measure of the efficiency of a column. Sometimes measured as  $N = 5.54(t_R/w_h)^2$ , where  $w_h$  (or  $w_{1/2}$ ) is the peak width at half height. See also *efficiency* and *theoretical plate*.

**v:** See *reduced velocity*.

**Narrow-bore column:** Columns of less than 2-mm i.d. used in HPLC. Also called *microbore*.

**$N_{\text{eff}}$ :** See *effective theoretical plates*.

**Nonaqueous reversed-phase chromatography:** Refers to reversed-phase chromatography performed without water as a component of the eluent on a reversed-phase packing. Used for very nonpolar compounds that cannot be eluted or are difficult to elute from a reversed-phase column with 100% methanol or acetonitrile. In these cases, solvent A should be acetonitrile, and solvent B should be a stronger solvent such as tetrahydrofuran. Reversed-phase rules apply to nonaqueous reversed-phase chromatography; that is, the more nonpolar the analyte, the greater the retention.

**Nonporous packing:** Particles similar to porous-layer bead but with particle diameters in the sub-5- $\mu\text{m}$  range; particles often are in the sub-2- $\mu\text{m}$   $d_p$  range. Used for high-speed separations in short columns.

Common column abbreviations include NPS, which refers to nonporous silica; NPR, which refers to nonporous resins; and NPZ, which refers to nonporous zirconia.

**Nonporous particle:** Refers to a solid particle used as a support for a porous coated or bonded phase; pellicular particles are nonporous particles of large particle diameter ( $\sim 40 \mu\text{m}$ ). Nonporous silicas and resins with small particle diameters of less than 3  $\mu\text{m}$  usually are microbeads with thin porous outer coatings of silica gel, bonded silica gel, or polymeric phase.

**Normal-phase chromatography:** A mode of chromatography performed when the stationary phase is more polar than the mobile phase. A typical normal-phase system would be adsorption chromatography on silica gel or alumina using mixtures of less polar eluents such as hexane–diethyl ether as a mobile phase. Also refers to the use of polar bonded phases such as cyano and alumina. Sometimes called straight-phase chromatography.

**Octadecylsilane:** The most popular reversed phase in HPLC. Octadecylsilane phases are bonded to silica or polymeric packings. Both monomeric and polymeric phases are available. Abbreviated in column names as C18 and ODS.

**Octylsilane:** A popular stationary phase in reversed-phase chromatography. Usually provides slightly less retention than the more popular C18. Both monomeric and polymeric phases are available. Abbreviated in column names as C8.

**ODS:** See *octadecylsilane*.

**On-column detection:** The column itself serves as the flow cell in HPLC or CE–CEC. Generally, the term used with fused-silica capillary applications. Outer polyimide layer is removed, an optical beam is directed through the capillary, and a measuring device such as a photomultiplier tube is located on the opposite side of the capillary.

**On-line preconcentration:** A precolumn is placed in front of the separation column to concentrate analytes before their separation. Different mechanisms — hydrophobic interaction, adsorption, or enzymatic reaction — may be used to retain analyte as a function of time. Then concentrated analytes are transferred to the separation column by a displacement process such as solvent elution or pH change.

**Open-tube capillary zone electrophoresis:** The application of CE principles in an

open capillary tube. Separations are based on differential electrophoretic mobility of charged compounds or ions. Typical capillary dimensions are 1–50 cm × 10–200 μm.

**Open tubular columns:** Small inner diameter columns (less than 100 μm) currently being investigated for use in HPLC, supercritical fluid chromatography (SFC), and CE. Stationary phases can be bonded on the internal walls of these small columns. The most frequently used column material is fused-silica tubing. Used very little in routine HPLC or SFC but frequently in CE.

**Optically active resin:** Incorporation of optically active groups into an ion-exchange resin to allow separation of optically active isomers. Few commercially available resins for HPLC applications.

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

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

## P

**Δp:** See *head pressure*.

**Pa:** See *pascal*.

**Packing:** The adsorbent, gel, or solid support used in an HPLC column. Most modern analytical HPLC packings are less than 10 μm in average diameter, and 5 μm is the current favorite.

**Paired-ion chromatography:** Same as *ion-pair chromatography*.

**Particle size ( $d_p$ ):** The average particle size of the packing in the LC column. A 5-μm  $d_p$  column would be packed with particles with a definite particle-size distribution because packings are never monodisperse. See also *monodisperse particles*, *particle size distribution*, and *polydisperse 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  $d_p \pm 10\%$  would mean that 90% of the particles fall between 9 and 11 μm for an average 10-μm  $d_p$  packing.

**Partition chromatography:** Separation

process in which one of two liquid phases is held stationary on a solid support (stationary phase) while the other is allowed to flow freely down the column (mobile phase). Solutes partition themselves between the two phases based on their individual partition coefficients. Liquid-liquid chromatography is an example; modern bonded-phase chromatography can be considered to be a form of partition chromatography in which 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. Also called distribution coefficient,  $K_D$ , and distribution constant ( $K_C$ ).

**Pascal (Pa):** A unit of pressure. 1 MPa is approximately 10 bar (atm) or 150 psi.

**Peak capacity ( $n$ ):** The number of equally well-resolved peaks ( $n$ ) that can be fit in a chromatogram between the holdup volume and some upper limit in retention. For  $R = 1$ ,  $n$  is given by the approximation  $1 + 0.25[(N)^{1/2} \ln(1 + k_n)]$ , where  $R$  is the resolution,  $N$  is the number of theoretical plates, and  $k_n$  is the retention factor for peak  $n$ .

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

**Peak doublet:** A split peak generally caused by a column void. Could be closely eluted compounds.

**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 Figures 1 and 2. See also *asymmetry*.

**Peak tracking:** A way of matching 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.

**Peak variance ( $\sigma^2$ ):** 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 Figure 2. See also *Gaussian peak*.

**Peak width ( $w_b$ ):** Same as *bandwidth*. See Figure 2.

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

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

**Perfusion chromatography:** Refers to chromatography performed using particles with very large pores (4000–8000 Å) called throughpores (megapores or gigapores). Eluent flows between the large pores and through the particles' 300–1000 Å interconnecting pores, called diffusive pores. Best suited for the preparative separation of macromolecules.

**Permeability ( $B_o$ ):** Also called column permeability and specific permeability. A term expressing the resistance of the packed column to the flow of mobile phase. For a packed column,  $B_o \approx d_p^2 \epsilon^3 / [180(1 - \epsilon)^2] = d_p^2 / 1000$ . A column with high permeability gives a low pressure drop.

**Permeation:** Refers to the SEC process in which a solute can enter a mobile-phase-filled pore of the packing.

**Phase ratio ( $\beta$ ):** 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. The retention factor is the product of the phase ratio and the partition coefficient.

**Phenyl phase:** A popular nonpolar bonded phase prepared by the reaction of dimethylphenylchloro- or alkoxyisilane with silica gel. Reportedly has affinity for aromatic-containing compounds and does impart a different selectivity compared with alkyl-bonded phases.

**Pirkle column:** Chiral, brush-type stationary phases based on 3,5-dinitrobenzoylphenylglycine silica used in the separation of a wide variety of enantiomers. Named after its developer, William Pirkle of the University of Illinois.

**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 *HETP*.

**Plate number:** See *column plate number*.

**Plate or plate number:** Refers to theoretical plates in a packed column (IUPAC). See also *theoretical plate*.

**Polyacrylamide gel:** Neutral hydrophilic polymeric packings used in aqueous SEC. Prepared by the copolymerization of acrylamide with *N,N'*-methylenebisacrylamide.

**Polydisperse particles:** Particles that have a substantial range of diameters (>10%).

**Polyethyleneimine:** An anionic polymeric phase used to coat or bond onto silica or a polymeric packing. Most often used for separating proteins and peptides.

**Polymeric packings:** Packings based on polymeric materials, usually in the form of spherical beads. Typical polymers used in LC are polystyrene–divinylbenzene (PS–DVB), polydivinylbenzene, polyacrylamide, polymethylacrylate, polyethylene-oxide, polydextran, and polysaccharide.

**Polymeric phase:** Refers to a chemically bonded phase in which a polymer species is bonded to silica-based particles.

**Polystyrene–divinylbenzene resin (PS–DVB):** 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 chromatography. Porosity and mechanical stability can be altered by varying the cross-linking through the DVB content.

**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 in nanometers. The pore size determines whether a molecule can diffuse into and out of the packing. See also *mean pore diameter*.

**Pore volume:** The total volume of the pores in a porous packing, usually expressed in milliliters per gram. More appropriately called the specific pore volume. It is measured by the BET method of nitrogen adsorption or by mercury-intrusion porosimetry in which mercury is pumped into the pores under high pressure.

**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 milliliters per gram.

**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 had 20–40  $\mu\text{m}$  particle sizes, which were larger than the microparticulate packings of today, but were easy to pack and provided adequate efficiency. Also called controlled surface-porosity supports and pellicular materials.

**Porous particle:** Refers to column packing particles that possess interconnecting pores of specified diameter and pore volume. For HPLC applications, analysts generally use porous particles with diameters less than 10  $\mu\text{m}$ . Larger particles are used in preparative-scale chromatography because of lower cost and higher column permeability.

**Porous polymer:** A packing material, generally spherical, that is based on organic polymers or copolymers. Popular examples include PS–DVB, polyacrylates, polydextrans, polyacrylamides, and polybutadienes.

**Precolumn:** A small column placed between the pump and the injector. It removes particulate matter that may be present in the mobile phase, presaturates the mobile phase with stationary phase or with dissolved substrate to prevent a loss of stationary phase or dissolution of the analytical column, and chemically absorbs substances that might interfere with the separation. Its volume has little effect on isocratic elution but contributes a delay to the gradient in gradient elution.

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

**Preparative chromatography:** Refers to the process of using LC 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 multiple grams of material. For isolating a few micrograms of valuable natural product an analytical column with a 4.6-mm i.d. can be used. Based on the intended need of the chromatographer, both size of columns are preparative chro-

matographic approaches.

**Pressure (pressure drop) ( $\Delta p$ ):** See *head pressure*.

**Pressure injection:** Pressure-induced injection in CE. Using pressure or vacuum to inject nanoliter-level volumes of sample into a capillary column. Best for narrow-bore capillaries that have inner diameters less than 10  $\mu\text{m}$ . A version of hydrostatic injection.

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

**Programmed-temperature chromatography:** Varying temperature during a chromatographic run. Seldom used in LC.

**PS–DVB:** See *polystyrene–divinylbenzene resin*.

**Pulsating flow:** Flow originating from a reciprocating pump. Normally, the pulses are dampened by a pulse damper, an electronic pressure feedback circuit, or an active damper pump head. Detectors such as electrochemical and refractive index detectors are greatly affected by flow pulsations.

**Q**

**Quaternary methyl amine:** A strong anion-exchange functionality popular in resin-based packings. Usually supplied in chloride form.

**Quaternary mobile phase:** A mobile phase comprising four solvents or buffers.

**R**

*r:* See *relative retention*.

**Radial compression:** Using radial pressure applied to a flexible wall column to reduce wall effects.

**Radial diffusion–dispersion:** Diffusion–dispersion across the LC 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 also radially, which allows the solute to reach the wall region where the eluent velocity is different than in the center of the column.

*Re:* See *Reynolds number*.

**Recovery:** The amount of solute or sample that is eluted from a column relative to the amount injected. Excellent recovery is important for good quantitation, prepara-

tive separations, especially biomolecules, and good peak shape and resolution. Reasons for inadequate recovery can be solute interaction with active sites on the packing, column frits, and column tubing. Compound decomposition during the separation process also can affect recovery.

**Recycling chromatography:** A technique in which the column effluent is recirculated onto the head of the column to take advantage of extended column length. Can be performed on a single column by passing the effluent through the pump again. 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 ( $h$ ):** Used to compare efficiencies of different columns;  $h = H/d_p$ , where  $H$  is the height equivalent to a theoretical plate and  $d_p$  is the particle diameter. An  $h$  value of 2 or less at the optimum velocity is considered to be a well-packed HPLC column.

**Reduced velocity ( $v$ ):** Used with the reduced plate height to compare different packed chromatographic columns. It relates the solute diffusion coefficient ( $D_M$ ) in the mobile phase to the particle size of the column packing ( $d_p$ );  $v = ud_p/D_M$ , where  $u$  is the average interstitial mobile-phase linear velocity. See also *Knox equation*.

**Refractive index peak:** A pseudo-peak normally found near the dead volume that results from the refractive index sensitivity of absorbance and other detectors. See also *vacancy peak*.

**Regeneration:** Regenerating 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 or 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 also can refer to bringing any column back to its original state; for example, removing impurities with a strong solvent.

**Relative retention ( $r$ ):** Retention relative to a standard;  $r = t_R'/t_{R(st)'} = k/k_{st}$ , where  $t_R'$  is the adjusted retention time of the component of interest,  $t_{R(st)'}$  is the adjusted retention time of the standard,  $k$  and  $k_{st}$  are the corresponding retention fac-

tors. For two adjacent peaks,  $\alpha$  expresses the relative retention and is called *separation factor* (formerly called selectivity or selectivity factor); calculated as  $\alpha = t_{R2}'/t_{R1}' = k_2/k_1$ , where  $t_{R2}'$  and  $t_{R1}'$  are the adjusted retention times of peaks 2 and 1, respectively, and  $k_2$  and  $k_1$  are the corresponding retention factors.

**Residual silanols:** The silanol ( $-\text{Si}-\text{OH}$ ) groups that remain on the surface of a packing after chemically bonding a phase onto its surface. These silanol groups, which may be present in very small pores, may be inaccessible to a reacting bulky organosilane such as octadecyltrimethylchlorosilane) but may be accessible to small polar compounds. Often they are removed by endcapping with a small organosilane such as trimethylchlorosilane. See also *endcapping*.

**Resin:** A solid polymeric packing used in ion-exchange separations. The most popular resins are PS-DVB copolymers with particle sizes less than 10  $\mu\text{m}$ . Ionic functionality is incorporated into the resin.

**Resolution ( $R_s$ ):** Ability of a column to separate chromatographic peaks;  $R_s = (t_{R2} - t_{R1})/[(w_{b1} + w_{b2})/2]$ , where  $t_{R2}$  and  $t_{R1}$  are the retention times of the two peaks and  $w_b$  is the baseline width of the peaks. It usually is expressed in terms of the separation of two peaks. A value of 1 is considered to be the minimum for a measurable separation to occur and to allow good quantitation. A value of 0.6 is required to discern a valley between two equal-height peaks. A value of 1.5 is considered sufficient for baseline resolution for two peaks of equal height. Values of 1.7 or greater generally are desirable for rugged methods. See Figure 2.

**Resolution equation:** Also called the general resolution equation and the Purnell equation;  $R = 4N^{1/2}[(\alpha - 1)/\alpha][k/(1 + k)]$ , where  $N$  is the efficiency,  $\alpha$  is the separation factor, and  $k$  is the retention factor.

**Retention factor ( $k$ ):** The period of time that the sample component resides in the stationary phase relative to the time it resides in the mobile phase. It is calculated from the adjusted retention time divided by the holdup time;  $k = (t_R - t_M)/t_M$ , where  $t_R$  is retention time for the sample peak and  $t_M$  is the retention time for an unretained peak. (Formerly,  $k'$  was used, and it was called the capacity factor or the capacity ratio.)

**Retention time ( $t_R$ ):** Also called the total retention time. The time between injection and the appearance of the peak maximum.

The total retention volume ( $V_R$ ) is determined by multiplying the retention time by the flow rate. The adjusted retention time ( $t_R'$ ) adjusts for the column void volume;  $t_R' = t_R - t_M$ . It usually is measured from the point of injection to the apex of the peak, but it should be measured to the center of gravity of the peak for asymmetric peaks.

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

**Reversed-phase chromatography:** The most frequently used mode in HPLC. Uses low-polarity packings such as octadecyl- or octylsilane phases bonded to silica or neutral polymeric beads. The mobile phase usually is 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. The technique has many variations in which various mobile-phase additives impart a different selectivity. For example, adding a buffer and a tetraalkylammonium salt to an anion analysis would allow ion-pairing to occur and generate separations that rival those of ion-exchange chromatography. More than 90% of HPLC analysts use reversed-phase chromatography.

**Reynolds number ( $Re$ ):** The ratio of viscous to inertial energy of the moving fluid. A measurement of flow in a smooth unpacked pipe;  $Re = ud/(\eta/\rho)$ , where  $u$  is the average velocity (in centimeters per second),  $d$  is the pipe diameter,  $\eta$  is the viscosity (in grams per centimeter seconds), and  $\rho$  is the density (in grams per cubic centimeters). At low  $Re$ , viscous friction dominates and controls fluid motion, making it slow and steady. In an unpacked tube, flow becomes fully turbulent when  $Re$  exceeds 4200. In a packed bed,  $u$  is replaced with the average interstitial velocity and  $d$  with the average particle diameter. Flow becomes turbulent in a packed bed at  $Re$  values greater than approximately 10 but is not fully turbulent until  $Re$  exceeds 100–200.

**$R_s$ :** See *resolution*.

**Running buffer:** The buffer system used to provide a conducting medium, which fills the capillary, and to participate in the

electrophoretic process.

**S** **S**: The solvent-strength parameter in reversed-phase chromatography. The solute-dependent slope of a plot of  $\log_{10} k$  versus volume fraction of organic modifier.  $S$  varies with modifier type, stationary phase, and temperature.

$\sigma^2$ : See *peak variance*.

**Salting-out effect**: Using a high-concentration 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 when proteins are precipitated first at high salt concentrations and then eluted by gradual dilution using reversed-gradient elution.

**Sample capacity**: Refers to the amount of sample that can be injected onto an LC column without overloading. Often expressed as grams of sample per gram of packing. Overloading is defined as the sample mass injected when the column efficiency decreases by 10% from its normal value; sometimes called sample loading.

**Sample stacking**: The sample solution is introduced at a low ionic strength — in pure water, dilute buffer, or organic solvent or mixtures of these compounds — and sandwiched between two portions of the buffer used in the CE separation. Because of the low ionic strength when a high voltage is applied to a capillary, sample ions move rapidly until they reach the CE buffer in which they slow down due to the lower field strength and thereby become concentrated and focused. Used for concentration sensitivity enhancement.

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

**SEC**: See *size-exclusion chromatography* and *steric exclusion chromatography*.

**Sedimentation**: A technique used for the sizing of resins for ion-exchange chromatography. A broad distribution of beads are 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. Workers can obtain very narrow cuts of particle size by sedimentation.

**Selectivity or selectivity factor ( $\alpha$ )**: Old term replaced by the *separation factor*. Sometimes called relative retention.

**Selectivity coefficient ( $k_{A/B}$ )**: In ion-exchange chromatography, the equilibrium coefficient obtained by applying the law of mass action to an ion exchanger and characterizing the ability of an ion exchanger to select two ions present in the same solution using electroosmotic flow. For example, the exchange of  $\text{Na}^+$  for  $\text{H}^+$   $k_{\text{Na}/\text{H}} = ([\text{Na}]_{\text{S}}[\text{H}]_{\text{M}})/([\text{Na}]_{\text{M}}[\text{H}]_{\text{S}})$ .

**Semipreparative chromatography**: Refers to preparative LC performed on analytical (4–5 mm i.d.) or slightly larger (6–10 mm i.d.) columns. Normal injection size would be milligram- to low-gram-size samples.

**Separation factor ( $\alpha$ )**: A thermodynamic factor that is a measure of relative retention of two substances. Formerly called selectivity or selectivity factor. The relative retention;  $\alpha = t_{\text{R}2}'/t_{\text{R}1}' = k_2/k_1$ , where  $t_{\text{R}2}'$  and  $t_{\text{R}1}'$  are the adjusted retention times of peaks 2 and 1, respectively, and  $k_2$  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 that normalize for both analysis time and pressure drop;  $E = t_{\text{R}}\Delta p/N^2v(1+k)$ , where  $t_{\text{R}}$  is the retention time,  $\Delta p$  is the pressure drop,  $N$  is the efficiency,  $v$  is the reduced velocity, and  $k$  the retention factor. The lower the value of  $E$ , the better the system.

**SFC**: See *supercritical fluid chromatography*.

**Silanol**: The Si–OH group found on the surface of silica gel. Silanols vary in strength depending upon their location, relationship to each other, and 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 or acidic silanol groups on a silica surface. Usually a strongly basic amine.

**Silica gel**: The most widely used HPLC packing. It has an amorphous structure, is porous, and is composed of siloxane and silanol groups. It is used in all modes of LC as a bare packing for adsorption, as the support for liquid–liquid chromatography or for chemically bonded phases, and as an SEC packing with various pore sizes. Microparticulate silicas of 3-, 5-, and 10- $\mu\text{m}$  average particle diameter are used in HPLC. Compared with irregular silicas, spherical silicas are preferred in modern

analytical HPLC columns because of their packing reproducibility and lower pressure drops. Sometimes called silica.

**Siloxane**: The Si–O–Si bond. A principal bond found in silica gel or a silylated compound or bonded phase. Stable, except at high pH values. Has little effect on the HPLC separation.

**Silylation**: The reaction process of an organochloro- or organoalkoxysilane with a compound that contains an reactive group. In LC, it refers to the process of derivatizing the solute before chromatography to make it detectable or to prevent unwanted stationary-phase interactions. It also can refer to the process of adding a chemically bonded phase to a solid support or deactivating the packing to reduce surface activity.

**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 and enable the continuous removal of product and reapplication of sample. A complex form of recycle chromatography used in preparative-scale chromatography.

**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 of approximately 10% (w/v) and rapidly pumped into the empty column using special high-pressure pumps.

**Snyder  $\epsilon^{\circ}$** : 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. Generates Type B silica gel with lower surface acidity, lower trace metal, lower surface area and porosity, and greater high-pH stability than older Type A silica gels.

**Solid-phase extraction (SPE)**: A technique for sample preparation using a 20–40  $\mu\text{m}$   $d_p$  solid-phase packing contained in a small plastic cartridge, disk, or in the wells of a 96-well flowthrough plate. The solid stationary phases used are identical to HPLC packings. Although related to chromatography, the principle of SPE is different and is sometimes called 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.

**Solid support:** Same as *support*.

**Solute:** The dissolved component of a mixture that is to be separated in the chromatographic column. See also *analyte* and *eluite*.

**Solvent:** The liquid used to dissolve a sample for injection into an HPLC column or CE capillary. Sometimes refers to the mobile phase used. See also *eluent*.

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

**Solvent selectivity:** Ability of a solvent to influence selectivity. For example, a change in solvent strength from 5% to 10% solvent B or a change from methanol to acetonitrile as the reversed-phase organic modifier will affect band spacing.

**Solvent-selectivity triangle:** A useful guide for choosing among different solvents for changing band spacing. Solvent selectivity is dependent on dipole moment, acidity, and basicity of the solvent molecule. See reference 4 for details.

**Solvent strength:** Refers to the ability of a solvent to elute a particular solute or compound from a column. Snyder described this quality for linear elution adsorption chromatography (liquid–solid chromatography) on alumina and quantitatively rated solvents in an eluotropic series. Less-extensive data are available for silica and carbon adsorbents. See also *Snyder  $\epsilon^\circ$* .

**Sorb:** The process of being retained by a stationary phase when the retention mechanism — adsorption, absorption, or partitioning — is unclear.

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

**SPE:** See *solid-phase extraction*.

**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 irregular particles often are used in preparative work because of their lower cost.

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

**Stationary phase:** The chromatographically retentive immobile phase involved in the chromatographic process. The stationary phase in LC can be a solid, a bonded, an immobilized or a coated phase on a solid support or a wall-coated phase. The stationary phase often characterizes the LC mode. For example, silica gel is used in adsorption chromatography and octadecylsilane bonded phase is used in reversed-phase chromatography.

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

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

**Steric exclusion chromatography:** 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*, and gel chromatography. Steric exclusion chromatography is used most often for polymer separation and characterization.

**Sterically protected bonded phase:** Bonded phase that has sterically protecting bulky functional groups such as isopropyl and isobutyl surrounding a siloxane covalent surface bond. Prevents attacks on siloxane bond, catalyzed hydrolysis, and loss of bonded phase at pH levels less than 3.

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

**Strong anion exchanger:** Anion-exchange packing with strongly basic ionogenic groups such as tetraalkylammonium groups.

**Strong cation exchanger:** Cation-exchange packing with strongly acidic

ionogenic groups such as sulfonate groups.

**Sulfonyl cation exchanger:** A strong cation-exchange functionality found in resin-based packings, usually propyl-SO<sub>3</sub>H. May come in cationic forms such as sodium, ammonium, silver, and calcium.

**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 that cannot be handled effectively by LC (because of detection problems) or GC (because of the lack of volatility). Examples include separations of triglycerides, hydrocarbons, and fatty acids. GC detectors and HPLC pumps have been used together in SFC.

**Superficial velocity ( $u_s$ ):** The hypothetical velocity that a mobile phase would have if the same column were operated unpacked but with the same flow rate;  $u_s = F/A_c$ , where  $F$  is the flow rate and  $A_c$  is the cross-sectional area of the tube.

**Superficially porous packing:** Same as *porous-layer bead*.

**Support:** Refers to solid particles. A support can be naked, coated, or have a chemically bonded phase in HPLC. Normally the solid support doesn't contribute to the chromatographic process.

**Suppressor column:** 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 membrane suppressors are used rather than a column.

**Surface area:** Refers to the total area of the solid surface in an adsorbent 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 less than 100 to 600 m<sup>2</sup>/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 the percentage of carbon is given as an indicator of surface coverage.

**Swelling–shrinking:** Process in which resins and gels increase or decrease their volume because of their solvent environment. Swelling is dependent upon the degree of cross-linking; low-cross-linking resins will swell and shrink more than highly cross-linked resins. If swelling occurs in a packed column blockage,

increased back pressure can occur, and column efficiency can be affected.

## T

**Tailing:** The phenomenon in which a normal Gaussian peak has an asymmetry factor greater than 1. The peak will have an extended trailing edge. Tailing is caused by packing sites 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 also can result from injecting an excessive mass or sample, badly packed columns, excessive extracolumn volume, poor fittings, excessive detector volume, and slow detector response. See Figure 1.

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

**Temperature programming:** Changing column temperature as a function of time during the separation. Rarely used in HPLC; if so, usually in a stepwise manner.

**Ternary mobile phase:** Mobile phase that is a mixture of three solvents or buffers.

**Theoretical plate (N):** A concept described by Martin and Synge. Relates chromatographic separation to the theory of distillation. Length of column relating to this concept is called height equivalent to a theoretical plate. See also *HETP*. Plates are calculated as  $N = 16(V_R/w_b)^2 = 16(t_R/w_b)^2$ , where  $V_R$  is the retention volume,  $w_b$  is the width at the peak base, and  $t_R$  is the retention time. See also **N**.

**Thermally tuned tandem column chromatography:** A form of LC in which two columns with distinctly different selectivities are placed in tandem and operated at two temperatures to optimize the resolution or analysis speed. Both columns use a common eluent, and the entire sample passes through both columns and is detected with a single detector. It is not a two-dimensional technique because each sample component provides only one peak.

**Titania:** An uncommon adsorbent used in adsorption chromatography.

$t_m$ : See *migration time*.

$t_M$ : Holdup time.

**Tortuosity or tortuosity factor:** A

packed-column property 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 tortuosity. See also *B term*,  $\gamma$ , and *molecular diffusion term*.

**Total mobile-phase volume ( $V_M$ ):** The total volume of mobile phase in an SEC column. Also known as totally included volume. Same as  $V_M$ .

**Total permeation volume ( $V_p$ ):** The retention volume of an SEC packing in which all molecules smaller than the smallest pore will be eluted. In other words, all molecules totally permeate all of the pores at  $V_p$  and are eluted as a single peak. Same as  $V_M$ .

**Total porosity ( $\epsilon_T$ ):** Ratio of the total volume of mobile phase in the column to the total column volume;  $\epsilon = V_M/V_c = \epsilon_c + \epsilon_i(1 - \epsilon_c)$ ; where  $V_M$  is the mobile-phase volume,  $V_c$  is the column volume,  $\epsilon_c$  is the interstitial porosity, and  $\epsilon_i$  is the intraparticle porosity.

**Totally porous packing:** The stationary phase is a porous matrix, and solutes penetrate the porous matrix to interact with the stationary phase.

$t_R$ : See *retention time*.

$t_R'$ : See *adjusted retention time* and *retention time*.

**Trace enrichment:** Technique in which 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 adding a stronger mobile phase in a concentrated form. The technique has been applied most successfully in the concentration of trace amounts of hydrophobic compounds such as polynuclear aromatic hydrocarbons from water using a reversed-phase packing. A strong solvent such as acetonitrile will elute the enriched compounds.

**Trailing electrolyte:** The strong electrolyte that contains the ion with the lowest mobility of any sample component ions in isotachopheresis.

**Transient isotachopheresis:** Separations in the zone electrophoresis mode performed by placing the capillary inlet in the leading electrolyte. When the leading electrolyte catches up with the sample zone, the gradient will be lost and CE separation will begin. The migration mode gradually changes from isotachopheresis to CE.

**Triethylamine:** A very common additive used to block silanol groups in reversed-phase chromatography when separating basic analytes.

**Trifluoroacetic acid:** A very common additive in reversed-phase chromatography for peptides and proteins.

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

**Turbulence:** The state in which fluid velocity fluctuates randomly at a point. See also *Reynolds number* and *turbulent flow*.

**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 what 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 under conditions using high Reynolds numbers. At these conditions, the  $H$  versus  $v$  curves show a decrease in  $H$  as  $v$  increases. See Figure 2.

$t_w$ : See *bandwidth*.

**Two-dimensional chromatography:** A procedure in which part or all of the separated sample components are subjected to additional separation steps. It can be performed by conducting a particular fraction eluted from the first column into a second column or system that has a different separation characteristic. It includes techniques such as two-dimensional TLC using two eluent systems in which the second eluent is applied after rotating the plate through 90°. It also includes LC followed by GC and one LC mode followed by a different mode such as reversed-phase chromatography followed by SEC. See also *multidimensional chromatography*.

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

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

$t_0$ : See *void time*.

## U

$u$ : See *linear velocity* and *velocity*.

$u_e$ : See *interstitial velocity*.

$u_M$ : See *mobile-phase velocity*.

$u_s$ : See *superficial velocity*.

$u_z$ : See *zone velocity*.

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

**van Deemter equation:** An equation used to explain band broadening in chromatography. The equation represents the height of a theoretical plate (HETP) and has three terms. The A term describes eddy dispersion or diffusion that results from axial velocity heterogeneity. The B term is for the contribution of molecular diffusion or longitudinal diffusion of the solute while passing through the column. The C term is the contribution from interphase mass transfer, which allows for the finite rate of transfer of the solute between the stationary phase and mobile phase. In its simplest representation,  $h = A + B/v + Cv$ . See also *reduced plate height* and *reduced velocity*.

**$V_c$ :** See *column volume*.

**$V_d$ :** See *dead volume*.

**$V_e$ :** See *interstitial volume*.

**Velocity ( $u$ ):** Same as *linear velocity*.

**$v_{eo}$ :** See *electroosmotic flow*.

**$V_i$ :** See *intraparticle volume*.

**Viscosity ( $\eta$ ):** Also called mobile-phase viscosity. The viscosity of the mobile phase varies with the temperature of the column. Low-viscosity mobile phases generally provide better efficiency than less-viscous ones because diffusion coefficients are inversely related to solvent viscosity. For example, column efficiency is higher in reversed-phase chromatography with acetonitrile as an organic modifier than with isopropanol, which is more viscous. Column back pressure is directly proportional to solvent viscosity.

**$V_M$ :** See *holdup volume*. Also mobile-phase volume.

**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 sometimes can be filled with glass beads or the same porous packing used in a column.

**Void time ( $t_0$ ):** The elution time of an unretained peak; also called the dead time and the holdup time ( $t_M$ ). The void volume is determined by multiplying the void time and the flow rate.

**Void volume ( $V_M$ ):** The total volume of mobile phase in the column; the remainder of the column is taken up by packing material. This volume can be determined by injecting an unretained substance. Also called dead volume. The symbol  $V_0$  is often used to denote the void volume. This is valid only for a column packed with nonporous particles.  $V_0$  is valid when used to denote the excluded volume ( $V_e$ ) in SEC.

**$V_p$ :** See *total permeation volume*

**$V_R$ :** See *retention volume* and *elution volume*.

**$V_R'$ :** See *adjusted retention volume*.

**$V_t$ :** See *total mobile-phase volume*.

**$V_o$ :** See *exclusion volume*.

**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 near the wall are carried along faster than the average of the solute band, and, consequently, band spreading results and the column loses efficiency.

**$w_b$ :** See *peak width*.

**Weak anion exchanger:** Anion-exchange packing with weakly basic ionogenic groups such as amino diethylamino ethyl groups.

**Weak cation exchanger:** Cation-exchange packing with weakly acidic ionogenic groups such as carboxyl groups.

**Wilke–Chang equation:** A semi-empirical equation used to estimate diffusion coefficients in liquids as a function of solute molecular size and solvent viscosity.

**X** **Xerogels:** Gels used in SEC that swell and shrink in different solvents. Also refers to silica-based packings that are prepared from acidification of soluble silicates to generate an amorphous, high-surface area, high-porosity, rigid particle.

**Z** **Zero dead volume:** Any fitting or component that has no volume that is unswept by the eluent.

**Zirconia:** Porous zirconium oxide. Used as a chromatographic sorbent, usually coated or bonded with polymeric organic phase.

**Zone:** See *band*.

**Zone velocity ( $u_z$ ):** The velocity at which the solute zone travels;  $u_z = u_M/(1 + k) = L/t_R$ , where  $u_M$  is the mobile-phase velocity,  $k$  is the retention factor,  $L$  is the column length, and  $t_R$  is the retention time.

**Zwitterions:** Compounds that carry both positive and negative charges in solution.

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