HICHROM

Chromatography Columns and Supplies

APPENDICES
Glossary of HPLC Terms

Catalogue 9

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GLOSSARY

Activity. In adsorption chromatography, the relative strength of the surface of the packing. For silica gel, the more exposed the silanol groups, the more active the surface. Activity can be controlled by adding water or another polar modifier, which is hydrogen-bonded to the active sites, thereby reducing the surface activity.

Additive. A substance added to the mobile phase to improve the separation or detection characteristics.

Adsorption. The process of retention in which the interactions between the solute and the surface of an adsorbent dominate.

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

Asymmetry (As). Asymmetry (skew) is a factor describing the shape of a chromatographic peak. Theoretically it is assumed peaks are symmetrical and of Gaussian shape. Practically, the peak asymmetry factor is measured as shown on p.14. A value of >1 indicates a tailing peak and <1 a fronting peak.

Back Pressure. The difference in pressure between the inlet and outlet of a column.

Bar. A unit of pressure equal to one atmosphere. It is equivalent to 14.5 pounds per square inch (psi) or 0.1 Megapascal.

BET Method. A method developed by Bruner, Emmett and Teller for measuring surface area. The level of liquid nitrogen adsorption within the pores of the phase is measured at very low temperatures. Pore volume and pore size distribution methods can also be obtained by this method.

Biocompatible. A term used to indicate that a tubing or fitting material will not change the biological activity of material coming into contact with it during the HPLC analysis time. It frequently means metal-free components.

Bonded. Term which implies that the stationary phase is chemically bonded to the surface of the supporting material.

Bonded Phase Coverage. Refers to the amount of bonded phase on a silica support. Coverage is usually described in $mmol/m^2$ or in terms of percentage carbon.

Capacity Factor (k′). An old term for a chromatographic parameter that measures the degree of retention (t_R). Now defined as the retention factor (k) by the IUPAC.

Channelling. Occurs when voids created in the packing material of a column may cause eluent and accompanying solutes to move more rapidly than the average flow velocity, resulting in band broadening. The voids are created by poor packing or by erosion of the packed bed.

Check Valve. A device built into an HPLC pump which allows the flow of eluent in one direction only.

Chiral Stationary Phase (CSP). Stationary phase designed to separate enantiomeric mixtures.

Column Dead-time (t_0). The time taken for solvent molecules or other non-retained peaks to move through the column.

Column Efficiency (N). A term used to express the width of a peak produced by a column. Efficiency is measured in terms of the number of plates, a parameter which is inversely related to the square of the peak width. See p.14 for the full calculation.

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

Dead Volume. A measure of solvent accessible volume between the injector and detector after the space occupied by the column packing material has been subtracted. Both interstitial column volumes and system (injector, detector, connecting tubing and end fittings) volumes contribute. The dead volume can be determined by injecting an inert compound (i.e. a compound that does not interact with the column packing) and measuring its retention volume.

Degassing. The practice of removing dissolved gases from the eluent. It can be achieved by helium sparging, applying vacuum to the eluent, ultrasonification or heating.

Denaturing. The process of destroying the tertiary and quaternary structure of a protein.

Desalting. A technique in which low molecular weight salts and other compounds are removed from non-ionic and high molecular weight compounds. An example is the use of size exclusion columns to exclude large molecules and retain lower molecular weight salts.

Dwell Volume. The volume between the point of mixing of solvents (usually in the mixing chamber or at the proportioning valves of the HPLC instrument) and the head of an LC column. Particularly important in gradient elution.

Eluotropic Series. A series of solvents with an increasing degree of polarity, generally used to explain solvent strength.

Elution Volume. Refers to the volume of eluent necessary to elute a solute from a column. For a symmetrical peak, it is the volume from the point of injection to the volume at maximum concentration.

GLOSSARY (continued)

Endcapping. The reaction of a silylating reagent with unreacted accessible silanols remaining on the silica surface after the initial bonding reaction. The process may reduce undesirable adsorption of basic or polar molecules which otherwise may cause peak tailing.

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

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.

Fractionation Range. In SEC, refers to the range in which the packing can separate molecules based on their size. Molecules that are too large to diffuse into the pores are excluded. Molecules that can diffuse into all of the pores totally permeate the packing, eluting unseparated at the permeation volume.

Fronting. A term describing a peak shape whose front has a leading edge.

Gel Filtration Chromatography (GFC). SEC carried out with aqueous eluents. It is sometimes referred to as aqueous GPC. Most gel filtration separations involve biopolymers.

Gel Permeation Chromatography (GPC). SEC carried out with organic eluents. Used for the separation and characterisation of polymers.

Gradient Elution. The process by which the strength and composition of the eluent is increased during the chromatographic run, thereby reducing analysis time. Binary, ternary and quaternary solvent gradients are routinely used.

Guard Column. A short column placed between sample injector and the inlet of the main column. It protects the analytical column against contamination from sample particulates and strongly retained solutes. The guard column is usually of cartridge format requiring a holder and packed with the same material as in the main column.

Helium Sparging. The process of bubbling helium through the eluent to remove dissolved gas.

High Pressure Mixing. A procedure in which two or more solvents are mixed on the high pressure side of the pumping system to form a final eluent. One pump is required per solvent.

Hybrid Silica. Silica gel comprising both organic and inorganic moieties with hybrid properties of polymeric packings and silica packings. Offers different selectivity but better high pH stability than bare or uncoated silica gel.

Hydrophilic. A description of compounds, solvents or bonded phases that either readily dissolve in water or prefer water to non-polar organic solvents, ie. 'water-loving'.

Hydrophilic Interaction Chromatography (HILIC). The use of polar stationary phases and partially aqueous eluents to separate compounds in order of increasing hydrophilicity (polarity).

Hydrophobic. A term describing compounds, solvents or bonded phases that dissolve easily in non-polar organic solvents such as hexane or prefer such solvents to water, ie. 'water-hating'.

Hydrophobic Interaction Chromatography (HIC). A protein separation technique in which reversed-phase materials are used with eluents containing high salt concentrations. Gradients are run by decreasing salt concentrations with time.

Hyphenated Techniques. Refers to the family of techniques best known by their acronyms, including LC-mass spectrometry (LC-MS), LC-Fourier transform IR spectroscopy (FTIR) and LC-MS/MS.

Injection Solvent. The solvent the sample is dissolved in prior to chromatographic analysis.

Interstitial Volume. The volume between the particles. It does not include the volume in the pores of the particles.

Ion Chromatography. 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.

Ion-Exchange Capacity. A measure of the number of ionic sites that can take part in the exchange process. Exchange capacity is expressed in mequiv/g.

Ion Exclusion. The process in which ionised solutes can be separated from non-ionised or partially ionised solutes using ion-exchange resins. Ionised solutes will move faster down the column.

Ion-Pair Chromatography. A form of reversed-phase chromatography in which ions in solution can be paired or neutralised prior to separation as an ion-pair. Ion-pairing reagents are usually ionic compounds that contain a hydrocarbon chain. The latter imparts a certain hydrophobicity to the resultant ion-pair allowing it to be retained on a reversed-phase column.

Ion Suppression. Buffering in an aqueous eluent at a particular pH to suppress solute ionisation. Useful for improving peak shape of weak acids and bases in reversed-phase chromatography.

GLOSSARY (continued)

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.

Isocratic. Chromatographic conditions in which a constant composition eluent is used.

Isoelectric Point. The pH point at which a molecule no longer has a net charge.

Ligand-exchange Chromatography. A technique in which chelating ligands are added to the eluent. On adsorption onto the stationary phase they act as chelating agents. An example is the use of copper amine chelates for the separation of amino acids.

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

Low Pressure Mixing. A pumping procedure in which two or more solvents are mixed on the low pressure side of the pump. Only one pump is required.

Mass Transfer. The process of solute movement between the moving and stationary zones. The faster the mass transfer process, the better the column efficiency. Mass transfer is represented by the C-term in the van Deemter equation.

Mean Pore Diameter. A term that refers to the average diameter of the pores within a phase.

Megapascal (MPa). A unit of pressure. One MPa equals about 10 bar (atmospheres) or 145 pounds per square inch (psi).

Modifier. A chemical added to reversed-phase solvent systems designed to optimise the chromatographic separation.

Monomeric Phase. A bonded phase in which individual molecules are bonded to a support. For silica, monomeric phases are typically prepared by the reaction of an alkyl- or aryl-monochlorosilane or alkoxysilane.

Nano LC. LC carried out with columns less than 100μm in internal diameter. Usually requires specialised instrumentation.

Overload. A saturation of the stationary phase by the solute which is evidenced by band broadening, tailing and flat edged chromatographic peaks.

Particle Size (dp). This term refers to the average particle size of the material packed into a column.

Particle Size Distribution. A measure of the distribution of the particles used to manufacture a column. In HPLC a narrow particle size distribution is desirable. For a 10μm size particle, a particle size distribution of dp±10% means that 90% of the particles have a 9-11μm size.

Peak Broadening. The tendency of a chromatographic peak to broaden as it passes through the column. It is also known as peak spreading or peak dispersion. The peak width or the number of theoretical plates in the column (N) is a measure of peak broadening.

Peak Capacity. The number of equally well-resolved peaks that can fit in a chromatogram between the hold-up volume and some upper limit in retention.

Polarity. A measure of the separation of charges within a molecule. Polar molecules interact more strongly with and are best separated on polar stationary phases.

Polymeric Packing. Packings based on polymeric materials, usually in the form of spherical beads. Common polymers include polystyrene-divinylbenzene, polymethylmethacrylate and polyvinylalcohol.

Polymeric Phase. A bonded phase in which typically a di- or trichlorosilane is reacted with more than one reactive silanol group on the surface of silica.

Pore Size (Mean Pore Diameter). The average diameter of the pore in a porous packing. 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. In SEC, the packings have different pore diameters, and therefore molecules of different sizes can be separated. For a typical adsorbent such as silica gel, 60Å and 100Å pore diameters are most popular. For packings used for the separation of biomolecules, pore diameters >300Å are used.

Pore Volume. The total volume of the pores in a porous packing, usually expressed in ml/g. It is measured by the BET method of nitrogen adsorption or by mercury intrusion porosimetry, where Hg is pumped into the pores under high pressure.

Precolumn. A column packed with silica placed between the pump and the injector. It presaturates the eluent with stationary phase minimising loss of the latter from the main column. It will also remove particulate material.

Pressure Drop. The difference in pressure between the inlet and outlet of a column during flow caused by the hydrodynamic resistance of the packed bed.

Process Scale Chromatography. Refers to the use of LC at the industrial scale level. Generally requires specially designed columns (eg. internal diameters > 5cm), recoverable solvents, low cost packings and overloaded operating conditions compared with laboratory scale HPLC.

Residual Silanols. These are the silanol (-SiOH) groups that remain on the surface of a silica after a bulky phase is chemically bonded to its surface. Their numbers can be reduced by further reacting (endcapping) the silica surface with a small organosilane.

GLOSSARY (continued)

Resolution (R_S). A measure of the separation of two adjacent peaks. The higher the resolution value the greater the separation (see p.370).

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 (see p.370).

Retention Time. The elapsed time between sample injection and the appearance of the chromatographic peak apex.

Sample Capacity. The term refers to the amount of sample that can be injected onto a column without overloading it. In preparative applications it is typically expressed as grams of solute per gram of stationary phase.

Scalability. In going from analytical to preparative chromatography, refers to the reproducibility of results on columns of different internal diameters and/or particle sizes when using the same bonded phase. A linear scale-up process minimises time required to optimise preparative separations.

Separation Factor (α). A thermodynamic factor that is a measure of relative retention of two substances. Formerly called 'selectivity' or 'selectivity factor'.

Siloxane Bond. The main –Si-O-Si- bond found in silica.

Size Exclusion Chromatography (SEC). A mode of HPLC used mainly to separate high molecular weight samples and to determine their molecular weight distribution by virtue of their size in solution. Also known as gel permeation, gel filtration or steric exclusion chromatography.

Superficially Porous Particle. A particle of typical diameter 2 to 5µm, with a solid core and a porous outer shell.

Surface Area. The total area of the phase's solid surface, as determined by an accepted measurement technique such as the BET method, which uses nitrogen adsorption. For silica it is typically 100-600 m^2/g.

Surface Coverage. Usually refers to the mass of stationary phase per unit area bonded to a chromatographic support. Often expressed in micromoles per square metre of surface. Sometimes the percentage of carbon is given as an indicator of surface coverage.

Tailing. The phenomenon in which a peak has an asymmetry factor >1. The downside of the peak will be skew.

Theoretical Plate. Measure of column efficiency. Length of column relating to this concept is called height equivalent to a theoretical plate (HETP).

Trace Enrichment. Technique in which trace amounts of compounds are retained on an HPLC or precolumn packing out of a weak eluent or solution and then are eluted by adding a stronger eluent, in a concentrated form.

Van Deemter Equation. 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. The B-term represents the contribution of molecular or longitudinal diffusion of the solute passing through the column. The C-term is the contribution from interphase mass transfer of solute between stationary phase and eluent.

Void. The formation of a space, usually at the head of the column, caused by a settling or dissolution of the packing. A void in the column leads to decreased efficiency and loss of resolution.

Void Volume (V_0). The total volume of eluent in the column, the remainder being taken up by packing material. Can be determined by injecting an unretained substance.

Zero Dead Volume (ZDV). It refers to a fitting or component which adds no extra volume to the system. In practice ZDV fittings have a finite but insignificant volume.

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