

Glossary

adsorption capacity Adherence of molecules in solution to cells, particles, or other molecules — or to solid surfaces, such as chromatography media.

affinity chromatography A separation method that uses the attraction of one molecule to another; immunoaffinity chromatography uses antibodies and metal affinity chromatography uses chelation for example.

affinity tags, affinity tails An amino acid sequence added to a protein to make purification easier; the tag's attraction to something is then used to separate that protein.

amino acid residues The portion of an amino acid left over after it has lost a water molecule in joining to another amino acid.

antibody An infection-fighting protein that tags, neutralizes, and helps destroy foreign microorganisms or toxins.

antigen A foreign substance that stimulates the immune system, then binds with an antibody.

asymmetry (A_s) The shape of a chromatographic peak; the distance between the peak apex and the back side of the curve over the front side of the curve; values greater than one are called *tailing peaks* and less than one are *fronting peaks*; used to confirm column packing and consistency.

anion-exchange (AEX) Anions are negatively charged atoms; they are attracted to positive sites; AEX resins are positively charged beads used for separating anions.

back flushing A column-switching technique in which a four-way valve placed between the injector and the column allows for mobile-phase flow in either direction; it is used to elute strongly held compounds at the head of the column either to analyze them or to remove them from the column.

band spreading, band broadening Bands are the different solutes separated by chromatography; smaller band widths means more efficient separations; bands spread out if fluid in the center of the chamber moves more rapidly than fluid that has diffused to the edges.

batch A specific, uniform quantity of a drug or solution produced during a single manufacturing cycle, using the same ingredient lots, manipulations, and processing time.

beads A sequence of beads offers resistance to the flow of the surrounding medium in a column; beads, for example, can be porous separating molecules by size, or they can carry a charge to separate ions and anions, among others; see *matrix and resin*.

bed Ion-exchange resin particles contained in a column; *bed depth* is the height of the resinous material in the column; *bed expansion* is when space increases between resin particles, which rise in the column. *Bed cracks* or *channels* are formed by *air bubbles*.

bed supports Solid elements at either end of a column that serve to contain the column packing; a porous framework within a column that supports adsorption; can be uncovered or coated or chemically bond a phase; made from stainless steel or other inert metal or plastic.

bubble trap Captures vapors and air bubbles, eliminating the need for a degassing *carrier* and preventing disruption to flow of fluid through a column; also called *gas lock*.

buffer A chemical used to maintain the pH of a solution by absorbing hydrogen (which would make the solution more acidic) or hydroxyl ions (which would make it more basic); *buffer capacity* is the ability of a buffer solution to absorb added alkali or acid.

cation-exchange Chemical process during which cations (positive charged ions) are

exchanged between solids and liquids; a form of ion-exchange chromatography.

chelation A process in which an organic chemical binds and removes free metal ions from solutions.

chemical compatibility, chemical resistance Withstanding change from chemical contact.

chromatography Separates molecules (usually proteins) based on how they bind to various solids, liquids, and gases, or based on their binding to a column lined with a ligand specific to a certain protein.

clarify, clarification steps Clearing a solution of turbidity (suspended particles).

clean-in-place (CIP) A method of cleaning large vessels (tanks, piping, and associated equipment) without moving them, using a high-pressure rinsing treatment; chemically cleaning and sterilizing equipment without moving it.

column A vertical, cylindrical container used to separate solutions by extraction, distillation, or chromatography; *column chromatography* uses the container to separate different components of a mixture as they migrate through a column.

column supports (tube flanges, tie rods, lifting fixtures) Used to support the stationary phase as near to the base of the column as possible to minimize dead space (where mixing of separated components could occur) below the column support.

conditioning tank A chamber in which a batch is preconditioned to humidity; suitable vessel for exposing batch to chamber atmospheric for vapor uptake.

compression factors Parameters for packing preparative liquid chromatography columns; in radial compression, pressure is applied to a flexible column wall to lessen *wall effects* (in which particles near walls are carried faster).

deadleg A stagnant section of piping.

dead space Volume outside of the column packing; the interstitial volume, including injectors, detectors, connecting tubing, and end fittings.

deerate Demonstrated removal of gas or air from a solution.

differential migration Chromatography is based on the different rates solutes in a liquid or gas move past adsorptive materials.

diluent A diluting agent added to a solution to increase volume and reduce concentration.

distributor Assembly that distributes fluid evenly into the column.

dynamic axial compression (DAC) Regulated pressure that eliminates the formation of voids or channels in packed beds, extending bed life.

eluent, elution profile eluate The separated components of a mixture that wash out from a chromatography column during elution; also called *elution fractions*.

eluate Separated solutes removed from a column.

eluent The substance used to recover samples from a column; also called an *elution solvent* or an *elution buffer*.

elution Washing out or removing adsorbed material in a column.

elution profile A graph that shows how much material is being carried out of the column over time; each *peak* represents a different separated material; also called a *chromatogram*.

end cells Flow distributors that provide a uniform flow across the entire cross sectional area of a column.

entrainment (air) Air pulled along by another substance; incorporation of air bubbles in bulk liquid; entrainment can be mechanical or by a sudden change in environment.

expanded-bed adsorption (EBA) A column fed from below with the *adapter* away from the packed resin, giving the resin room to expand, creating spaces between the beads.

feed, feedstock, feed stream Raw broth containing particles to be removed by a column; the solution fed into a column for separation.

finer Loose particles from column packing material, that can clog a column outlet filter; fines in trace amounts may not affect the separation, but fines can occlude the bottom screens or *frits*, creating *backpressure*, which would require a reduced *flow rate*, reducing column *throughput*.

flow, flow rate Movement of solution through a column with a continual change of place among the constituent particles; flow is affected by the column *packing*; *flow rate* affects a column's pressure, separation quality, and analysis time; *laminar flow* is a parallel, uniform, nonturbulent flow of a gas or liquid, with little mixing and no obstruction or bends.

fractionation Any laboratory method which is used to separate the components of a mixture; *fraction* usually refers to the protein of interest; *fractionation range* is the pore size of the filters in gel filtration media.

frits The steel, plastic, or PTFE material at either end (or often actually in the *end fitting*) of a column, containing the column packing.

fronting *Peak* shape is the front pan of a peak (before the apex); *tailing* is the back pan; see *asymmetry*.

gel A semirigid solid, almost jelly-like; *gel-filtration chromatography* separates proteins by running the protein solution through a column filled with porous gel beads that trap or slow down small molecules, but that allow larger molecules to slide past.

headspace The volume above the solution within a column.

heat exchangers A mechanical device designed to transfer heat energy from one medium to another.

height equivalent to theoretical plate (HETP) A measure of column efficiency; degree of *flow* without *band broadening*; discrete bands are called *theoretical plates*.

hydrophobic-interaction chromatography (HIC) Hydrophobic ("water-hating") substances don't dissolve well in water; used to separate hydrophobic proteins using a high salt medium.

immunoglobulins Infection-fighting proteins; also called *antibodies*.

in situ Something in its original place; without removal to a laboratory.

interferon A glycoprotein in animal cells that inhibits virus reproduction and affects cell development (differentiation).

ion-exchange chromatography (IEC) Separation using anion (negative) or cation (positive) packing.

jacket A temperature control method using liquid or steam flowing through the jacket to heat (or cool) the fluid in the vessel.

leaching Removal of soluble constituents by percolating liquid; separating soluble components; leachate is a solution of material removed from a solid.

marker A characteristic that identifies a cell or molecule or makes it recognizable.

media Plural form of medium; Any substance in which chromatographic separation occurs.

mobile phase A medium that carries a sample; the solvent that moves the solute through the column.

pack, packing The adsorbent, gel, or solid used in a column.

peaks The sharp rise in a chromatogram; *peak shape* is the profile of a chromatographic peak; see *asymmetry*.

pressure equipment directive (PED) The PED was implemented in the European Union in May 2002; under the PED, manufacturers using the ASME code cannot be certain about continued market access.

plasticizers An agent, usually organic, added to a stiff or brittle substance to make it soft and flexible.

plug flow moving material as a unit, without dissolving or shearing

purification A central part of downstream processing that takes a crude solution and isolates the product from it in a fairly pure form.

qualification Documenting that a piece of equipment does what it was designed to do, was installed correctly, and continues to operate within specified parameters over time.

resin Used generically to describe chromatographic media, particularly polymer beads; a polymer that is used as a matrix.

resolution Amount of distinguishability of a mixture component; the quality of a chromatographic separation measured by the purity of the resulting component fractions.

scale-up To take a manufacturing process from the laboratory to a commercial scale.

sintered To make a coherent mass by heating, without melting.

size-exclusion chromatography (SEC) A separation method that uses porous particles to separate molecules of different sizes.

slurry A thin semifluid suspension of a solid in a liquid; a *slurry packing system (SPS)* is the technique most used to pack HPLC columns with microparticles.

solute A substance that is dissolved in a solvent; the part of a solution that is uniformly dispersed in another substance.

stationary phase The solid, nonmobile component in chromatography; the *mobile phase* flows over or through the stationary phase to effect the separation.

viscosity Thickness of a liquid; determines its internal resistance to shear forces.

voids Spaces created by poor packing or erosion that can cause the *mobile phase* and accompanying solutes to move more rapidly than the average flow velocity, resulting in *band broadening*.

wall effect If packing is looser near the walls of a rigid column, the mobile phase can flow faster because of the decreased permeability; solute molecules near the column wall are carried along faster than the average, which results in band spreading.