Resins Selection Guide

MilliporeSigma is the U.S. and Canada Life Science business of Merck KGaA, Darmstadt, Germany.

Supelco®
Analytical Products

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Supelco® LC Media

Low pressure liquid chromatography (LPLC) is the oldest of chromatographic techniques, originally performed with open columns that use gravity to move the sample through the packing bed. Now, pumps and other instrumentation have been incorporated into LPLC and superior packing media have been developed specifically for this versatile technology. We offer a broad selection of media, packed columns, empty columns, filters, injectors, valves, and tubing for LPLC.

The final pages of this guide describe Carboxen® Synthetic carbons. The synthetic carbons are highly engineered derivatized resins with function in both adsorption and hydrophobic interaction chromatography.

Key Words:

- low pressure liquid chromatography
- adsorption chromatography
- affinity chromatography
- gel filtration chromatography
- hydrophobic interaction chromatography
- ion exchange chromatography

Low pressure liquid chromatography (LPLC), formerly referred to as liquid chromatography, is one of the oldest chromatographic techniques. At its inception, liquid chromatography was performed in open columns using gravity to "pump" the solvent through the column. This type of LPLC is also called open column liquid chromatography, because the top of the column is not sealed with an end-fitting, but instead is connected to a solvent reservoir. To introduce a sample onto the column, the solvent is drained to the top of the packing bed and the reservoir is removed. The sample is first added via a pipette and then, the solvent carefully, ensuring that the sample band is not disturbed. The solvent reservoir is finally reattached.

Over the past decades, instrumentation has been incorporated into LPLC. An inexpensive low pressure pump (often peristaltic) feeds the solvent onto the column. The sample can be introduced through an injector (low pressure), as in HPLC, and a simple UV or RI detector can be placed in line to monitor the column effluent. In yet another variation that is popular with organic chemists - flash chromatography - an inert gas is employed to force the solvent through the column.

LPLC Modes

This guide is organized alphabetically by the respective chromatographic modes: adsorption, affinity, gel filtration, hydrophobic interaction, and ion exchange. Media for reversed phase LPLC are included with the adsorption media. Synthetic carbons are covered in the final pages of this guide.

Packing Materials (Media)

Although some LPLC columns are available in already-packed form, more often the analyst fills a column with the packing material of choice, depending on the LPLC application. Traditional inorganic adsorbents include silica and bonded silica, alumina, carbon, and Florisil® gel. The most important use for inorganic matrices is in the purification and isolation of small molecular weight compounds, including environmental analytes. Polymeric and Synthetic Carbon adsorbents have important applications in the pharmaceutical industry, for e.g., purification of antibiotics from fermentation broths. These adsorbents' stability over virtually the entire pH range, makes them easy to clean with caustic solutions. Polymeric ion exchangers are predominantly used for water softening and polishing, chelation, metal processing, acidification, neutralization, decolorization, etc.

Biochemical research has spawned the development of additional media. Dextran-based Sephadex™ beads were among the first media biochemists used to purify proteins for gel filtration and ion exchange applications. These, and other soft-gel beads, such as Sepharose® and Sephacryl™ media, have been important factors in the continuous improvements in protein purification since the late 1950s. Other developments include media with better pressure stability and resolution (e.g., Toyopearl® and Superdex™ media) to allow higher flow rates and reduced purification times. Carboxen Synthetic carbon media are designed to withstand high pressures, up to 110 MPa and have excellent flow characteristics. Resins media undergo plastic deformation at far lower pressures. The synthetic carbons have demonstrated excellent clearance of high-risk impurities in biochemical purifications.

Column Hardware, LPLC Accessories

Glass and inert plastic are the most common column materials. Depending on what solvents come into contact with the column hardware, the fittings are either made from polypropylene (for aqueous mobile phases) or materials that can resist organic solvents, such as Teflon® or KEL-F® polymers.

Empty columns of various design, fittings, injectors, valves, filters, and tubing suitable for low pressure liquid chromatography are listed on **SigmaAldrich.com**.

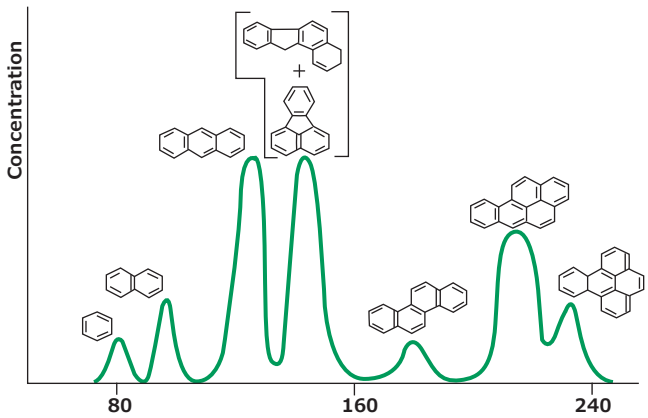
Adsorption Chromatography

Adsorption and partition chromatography are used in a wide variety of synthetic and biochemical separations. Adsorption chromatography relies on nonspecific dipole-dipole interactions between the packing and the analyte(s). Separation by partition chromatography is based on differences in the solubilities of the sample components in the mobile and stationary phases.

Our selection of adsorption and partition chromatography media includes alumina, carbon, hydrophobic dextran, Florisil®, polymeric adsorbents, and silicas and bonded phase silicas. Media are listed below in alphabetical order by type.

PAHs on Sephadex™ LH-20

Packing:	Sephadex™ LH-20
Cat. No.:	LH20100-50G
Column:	1.1 x 112 cm
Mobile Phase:	isopropanol
Flow Rate:	2.5 mL/hr
Inj.:	0.25 mL (solvent: isopropanol)



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Adsorption Media

Medium	Forms & Brand Names	Potential Applications
Alumina, Activated 150 mesh	Bulk packing, Solid phase extraction tubes	Catecholamines; vitamins; natural alkaloids; glycosides; water-soluble dyes; peroxides; extraction of polar compounds
Carbon 20-50 mesh, 50-100 mesh	Bulk packing, ORBO™ tubes	Toxic emissions in air; ultrapure water preparations; liquid/vapor phase separations pharmaceuticals, precious metal scavengers
Dextran, Hydrophobic 10-23 µm, 25-100 µm	Bulk packing, hydroxyalkoxypropyl dextran, Sephadex™ LH-20	Varying hydrophobicities. Gel permeation chromatography; lipids; steroids; fatty acids; vitamins
Florisil® (Magnesium Silicate) <74-1200 µm 150-250 µm (PR)	Bulk packing, ORBO™ tubes, Solid phase extraction tubes	Pesticide analyses; PCBs; herbicides; phenols; polar compounds; steroids; antibiotics; vitamin assay; decolorization
Polymers (Polystyrene/Divinylbenzene most 20-60 mesh, some 100 µm	Bulk packing, ORBO™ tubes, Solid phase extraction tubes Amberlite™ XAD™, Amberchrom™, Diaion™, Amberlite™, Supelite™ DAX-8, Supelpak™	Surfactants; pharmaceuticals; environmental analysis; organics; wastewater treatment; pesticides; humic substances; vitamins; decolorization
Silica purified: 15-650 µm, 20-150 Å bonded RP phases: 20-63 µm, 60 Å, 300 Å	Bulk packing Davisil®, Supelco®	Flash chromatography; cleanup and purification of a wide range of synthetic and natural compounds

Affinity Chromatography

Affinity Media

We offer a large variety of affinity media, in bulk or prepacked columns.

A successful affinity separation requires a biospecific ligand covalently attached to a chromatographic bed material, the matrix. Properties of affinity matrices are presented in the table below. These properties will differ with the ligand bound. For example, the Toyopearl® Epoxy media are used at pH 9-11, 40 °C to make a stable secondary amine linkage, while at pH 7-8, 25 °C, they form a stable sulfide linkage.

Affinity Matrices

Matrix	pH Range	Max. Operating Pressure & Flow	Properties/Limitations
Acrylic Beads 150 µm	cleaning: 0-12 operating: 5-9	0.2 MPa, 100 mL/min (2 x 28 cm column)	Macroporous; stable in organics; hydrophilic, neutral; no shrinking/swelling with changes in ionic strength; mechanically stable; high binding capacity
Agarose, Beaded 2%: 60-200 µm 4%: 45-165 µm 6%: 45-165 µm	4-9	2%: 0.04 MPa, 0.8 mL/min 4%: 0.08 MPa, 1.0 mL/min 6%: 0.20 MPa, 1.2 mL/min (2.5 x 30 cm column)	Analytes up to 4 x 10^7 Da; temperature to 40 °C; sterilizable (chemical); insoluble in all common solvents; should not be used with oxidizing agents or chaotropic salts
Cellulose fibrous tubes, 30-60 µm diam., 85-250 µm long	2-10	0.07 MPa, 2.4 mL/min (2.5 cm column diameter)	Low resolution; pliable material
Sepharose® 2B: 60-200 µm 4B: 45-165 µm 6B: 45-165 µm	4-9	2B: 0.04 MPa, 0.8 mL/min 4B: 0.08 MPa, 1.0 mL/min 6B: 0.20 MPa, 1.2 mL/min (2.5 x 30 cm column)	Analytes up to 4 x 10^7 Da; temperature to 40 °C; sterilizable (chemical); insoluble in all common solvents; should not be used with oxidizing agents or chaotropic salts
Sepharose® CL 2B: 60-200 µm 4B: 45-165 µm 6B: 45-165 µm	3-14	CL-2B: 0.05 MPa, 1.2 mL/min CL-4B: 0.12 MPa, 2.2 mL/min CL-6B: 0.20 MPa, 2.5 mL/min	Analytes up to 4 x 10^7 Da; high flow rates; sterilizable (autoclave/chemical); insoluble in all common solvents; can be used (2.5 x 30 cm column) in dissociating media, high concentrations of chaotropic salts
Toyopearl® 40-90 µm	operating: 2-12 cleaning: 1-13	0.7 MPa	Analytes up to 1 x 10^6 Da; 1000 Å pores; hydrophilic, neutral; compatible with solvents; chemically resistant; volume stable to changes in pH or ionic strength

80 cm water= 0.8 bar = 11.6 psi = 0.08 MPa.

Affinity Ligands

The ligand should exhibit specific and reversible binding affinity for the substance to be purified. In addition, the ligand should possess chemically modifiable groups that allow attachment to the matrix without destroying its ligand-binding activity. The dissociation constant (Kd) for the ligand-binding substance complex should ideally be in the range of 10^{-4} to 10^{-8} M in free solution. If no information is available on the strength of the binding complex, use a trial and error approach. It is important to consider the region of the ligand that will be used for attachment to the matrix. If several functional groups are available, the ligand should be coupled via the group least likely to be involved in specific interaction with the molecule to be isolated.

Spacer Arms

The active site on a biological molecule is often located deep within the molecule. Adsorbents prepared by coupling small ligands directly to the matrix can exhibit low capacities, due to steric hindrance. To prevent this effect, a spacer arm can be used between the matrix and ligand, to facilitate effective binding. Alternatively, if the spacer arm is too long, nonspecific effects become pronounced and reduce the selectivity of the separation.

Affinity Chromatography, continued

Affinity Media

Group-specific media have affinity for a group of related compounds, rather than for a single substance, thus enabling the analyst to use the same general ligand to purify several substances (e.g., a class of enzymes). Within the group, there is either a structural or functional similarity. For example, heparin-bearing affinity media recognize β-pleated sheet domains. Thus, heparin media are useful for purifying coagulation factors, lipoproteins and lipoprotein lipases, growth factors, and enzymes active in nucleic acid metabolism.

Activated media are resins with activated functional groups ready for direct coupling of a protein or other ligand. The cyanogen bromide-activated matrices are typical examples. The reaction of cyanogen bromide with the matrix activates the product, enabling its coupling with proteins, nucleic acids, or other biopolymers, under mild conditions, via primary amino or similar nucleophilic groups.

Resins with reactive groups employ carbodiimide coupling or reductive amination to achieve covalent bonding. Aldehyde groups may be present in a carbohydrate or glycoprotein ligand or can be introduced into the intended ligand by mild periodate oxidation. This reactive matrix is used for coupling either proteins or low molecular weight ligands (e.g., lactose).

Affinity Terminology

Matrix: solid support to which ligand is attached

Activation: chemistry by which ligand is bound to matrix

Attachment: position on ligand through which activated matrix is bound

Spacer: identity or chain length of molecular fragment between activated matrix and ligand (including activating group)

Ligand Immobilized: quantity of ligand per mL of resin

Binding Capacity: saturating amount of a specific compound reversibly bound per mL of resin

Swelling: if resin is sold on a weight basis, approximate swelling ratio (grams to milliliters) is given for convenience. A lot-specific value is given for most products on their labels, as an aid in preparing columns of predetermined size

Applications for Affinity Media

Affinity Class	Potential Applications
Activated	Functional spacer; support matrix; eliminates handling of toxic reagents
Amino Acid	Serum proteins; proteins; peptides; enzymes; rRNA; dsDNA
Avidin & Biotin	Purification of biotin/avidin & derivatives; biotinylated substances. Biotin derivatives dissociate under nondenaturing conditions.
Carbohydrate	Glycoproteins; lectins; other carbohydrate metabolite proteins. Proper selection can ensure one-step purification.
Dye	Nonspecific interaction. Mimic biological substances (substrates, cofactors, effectors); proteins. Optimize purification protocol with different dyes.
Glutathione	Detoxification enzymes; glutathione-S-transferase and fusion proteins; glutathione peroxidase and glyoxalase
Hydrophobic	Couple ligands containing free carboxyl groups; proteins
Immunochemical	Removal of antibodies from antisera or serum proteins. Solid phase second antibodies.
Lectin	Soluble glycoproteins; other carbohydrate-containing substances
Nucleotide/ Coenzyme	Dehydrogenases; kinases; transaminases. Reliable adsorbents.
Nucleic Acid	mRNA; DNA; rRNA; other nucleic acids and oligonucleotides
Specialty	Purification of specific classes or types of proteins, coenzymes, or physiological partners
Toyopearl® Resins	
Group Specific	Large pore diameter; medium pressure Nucleotide-dependent enzymes; bind histidine and free cysteines of peptides or proteins; purify coagulation factors; lipoproteins; enzymes active in nucleic acid metabolism.
Activated	Large pore diameter; medium pressure; optimal pH 7-9; highly reactive to amine/thiol groups. Immobilize protein or low molecular weight ligands.
Reactive	Large pore diameter; medium pressure; pH range 6.9-9 or 4.5-6. Primary amines; carboxylate, aldehyde, or amino groups.

Recommended Reading

Affinity Chromatography: Principles & Methods

Cytiva, 1993, 143 pp.

This handbook serves as an introduction to affinity chromatography and a practical guide to the media developed by Cytiva. The text covers ligands, spacers, and coupling gels, and describes general experimental methods.

Immobilized Affinity Ligand Techniques

G.T. Hermanson, A. Krishna Mallia, and P.K. Smith, Academic Press, 1992, 454 pp.

A practical guide to the preparation and use of immobilized affinity ligands for purification, catalysis, and analysis. Special emphasis is on immunochemical techniques, including antibody isolation, preparation of antibody fragments using immobilized enzymes, and immunoaffinity chromatography. Describes processes for making optimized affinity supports.

Gel Filtration Chromatography

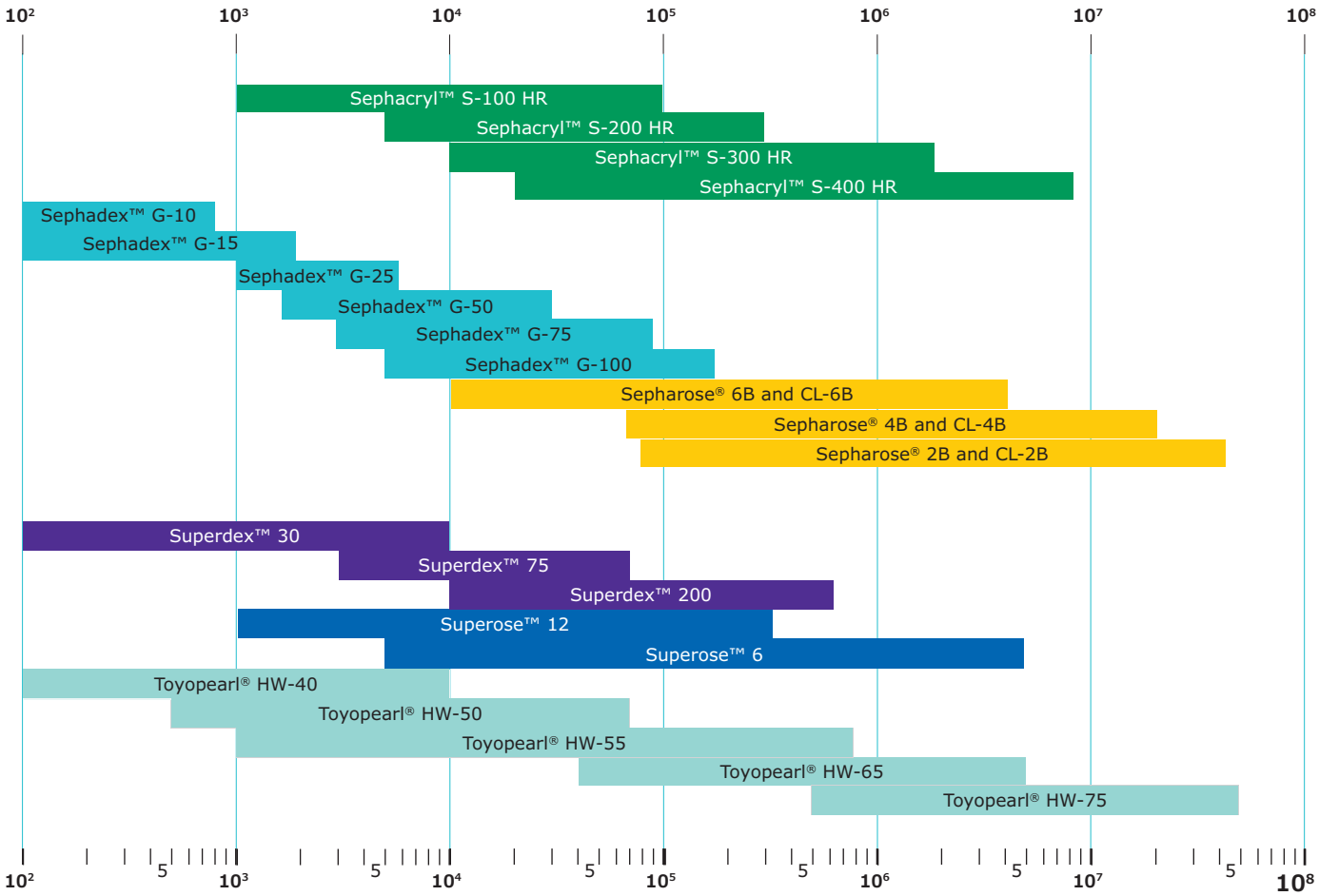
In gel filtration and gel permeation chromatography - also known as size exclusion chromatography - separation is based on differences in the size and/or shape of the analyte molecules, which governs the analytes' access to the pore volume inside the column packing particles. The exclusion limit of a size exclusion packing indicates the molecular weight, for a particular polymer type, above that analytes are fully excluded from entering the pores and thus will not be separated. According to their sizes, smaller analytes have partial to complete access to the pore volume. Among the analytes that partially or fully enter the pore volume, larger molecules with lesser access to the pore volume elute first, while the smallest molecules elute last. The fractionation range identifies the molecular weight range from the largest molecule that is fully included to the smallest molecule that is fully excluded.

The Supelco® portfolio offers gel filtration media for low and medium pressure liquid chromatography. Packing materials are supplied with instructions for packing columns, for use with gravity feed or low to medium pressure pumps. Available media include those manufactured by Cytiva and Tosoh Corporation.

Media selection charts in this guide contain pertinent information about each gel filtration packing material. Organized alphabetically by media names, the charts list chemical composition, exclusion limits, pH and pressure stability, and particle size ranges. Key properties, limitations, and potential applications also are listed.

Refer to the adsorption media listings in the website for Sephadex™ LH-20, used for purifying small molecular weight compounds by gel permeation and partition chromatography. Hydroxyalkoxypropyl dextran is also listed with the adsorption media.

Molecular Weight Ranges for Gel Filtration Media



Gel Filtration Chromatography, continued

Gel Filtration Media

Medium	Fractionation Ranges (proteins, Da)	Properties/Limitations	Potential Applications
Sephacryl™ Matrix: acrylic (dextran/ bisacrylamide copolymer) 25-75 µm S-1000: 40-105 µm 0.2 MPa (2.6 x 30 cm column) pH range: 2-11	S-100 HR: 1K-100K S-200 HR: 5K-250K S-300 HR: 10K-1500K S-400 HR: 20K-8000K S-500 HR, S-1000: ND	Clean with 0.2 M sodium hydroxide, 0.1 M hydrochloric acid, 1.0 M acetic acid Compatible with detergents, chaotropic salts, dissociating agents Sterilize by autoclaving Compatible with organic solvents Scaleable Good recoveries Stable bed volume	S-100 HR: peptides, small proteins S-200 HR: proteins, some small serum proteins (e.g., albumin) S-300 HR: membrane & serum proteins, monoclonal antibodies S-400 HR: large proteins & macromolecules with extended structures (e.g., proteoglycans, liposomes) S-500 HR: large macromolecules, small particles (e.g., plasmids)
Sephadex™ Matrix: dextran (crosslinked with epichlorohydrin C: 100-300 µm M: 50-150 µm F: 20-80 µm SF: 10-40 µm SF: 0.016-0.160 MPa (2.6 x 30 cm column) pH range: 2-13 (< G-25) 2-10 (> G-25)	G-10: < 700 G-15: < 1500 G-25: 1K-5K G-50: 1.5K-30K G-75: 3K-80K G-100: 4K-150K	Classic support Swelling 2-40X Use LH grades for organic solvents SF grade for best resolution, low flow rate F grade for prep lab work M grade for standard lab work C grade for very crude samples Can be autoclaved at 120 °C salts, dissociating agents Clean with 0.2 M sodium hydroxide Ideal for molecular weight determinations Interact with aromatic compounds	G-10: very low molecular weight substances; desalting; buffer exchange; peptides G-15: low molecular weight compounds; interact with aromatic compounds; desalting; buffer exchange; peptides G-25: medium grade available in packed PD-10 columns for rapid, routine desalting; interact with aromatic compounds; small peptides & proteins; buffer exchange G-50: desalting; buffer exchange; standard for process scale; very low nonspecific interactions G-100: very low nonspecifc interactions
Sepharose® Matrix: agarose 6B: 45-165 µm 4B: 45-165 µm 2B: 60-200 µm 0.004-0.02 MPa (2.6 x 30 cm column) pH range: 4-9	6B: 10K-4000K 4B: 60K-20,000K 2B: 70K-40,000K	Clean with 0.5 M sodium hydroxide Good recoveries Broad fractionation range Temperature range: 4-40 °C Cannot be autoclaved Incompatible with oxidizing agents, chaotropic salts, dissociating agents	2B: DNA-protein complexes; viruses; asymmetric molecules; affinity support 4B: tRNAs; membrane proteins; polysaccharides; affinity support 6B: polio virus purification; proteins; polysaccharides; affinity support

80 cm water = 0.8 bar = 11.6 psi = 0.08 MPa

Medium	Fractionation Ranges (proteins, Da)	Properties/Limitations	Potential Applications
Sepharose® CL Matrix: agarose (Sepharose® reacted with 2,3-dibromopropanol) 6B: 45-165 µm 4B: 45-165 µm 2B: 60-200 µm 0.005-0.02 MPa (2.6 x 30 cm column) pH range: 3-14	6B: 10K-4000K 4B: 60K-20,000K 2B: 70K-40,000K	Stable in chaotropic salts, dissociating agents Flow 2X that of Sepharose® Sterilize by autoclaving Easy switch to organic solvents Good recoveries Broad fractionation range Ideal for long-term routine applications Do not use with oxidizing agents	2B: very high molecular weight molecules (e.g., nucleic acids); polysaccharides; cell particles; viruses 4B: vesicles; membrane proteins; polysaccharides; affinity support 6B: viruses; proteins; polysaccharides; affinity support.
Superdex™ Matrix: dextran (covalently bonded to agarose) 24-44 µm 0.5 MPa pH range: 3-12	30: ≤10K 75: 3K-70K 200: 10K-600K	Clean with 1 M sodium hydroxide, 0.1 M hydrochloric acid, 1.0 M acetic acid Can be used with detergents, chaotropic salts, dissociating agents Sterilize by autoclaving Compatible with organic solvents Scaleable High recoveries Highest selectivity	30: peptides; oligosaccharides; small proteins. Selectivity between Sephadex™ G-25 & G-50 75: high-resolution prep separations; wide range of recombinant DNA products. Selectivity similar to Sephadex™ G-75 200: monoclonal antibodies; high-resolution prep separations; contaminants of lower molecular weight (albumin, transferrin); good when protein molecular weight is unknown. Selectivity similar to Sephadex™ G-200
Superose™ Matrix: agarose (highly crosslinked) 20-40 µm 12: 0.7 MPa 6: 0.4 MPa (1.6 x 50 cm column) pH range: 2-14	12: 1K-300K 6: 5K-5000K	Clean with 0.2 M sodium hydroxide, 0.01 M hydrochloric acid, 1.0 M acetic acid Compatible with detergents, chaotropic salts, dissociating agents Sterilize by autoclave Compatible with organic solvents FPLC-HiLoad Some hydrophobic interactions	Preparative work; proteins; DNA fragments; polysaccharides
Toyopearl® HW Matrix: methacrylate (ethylene glycol/ methacrylate copolymer) C: 50-100 µm F: 30-60 µm S: 20-40 µm 0.7 MPa pH range: 2-12	40: ≤10K 50: 500-80K 55: 1K-700K 65: 40K-5000K 75: 500K-50,000K	High mechanical strength (5-7 bar) Stable in organics Surfactant, dissociating agent, acid/base cleaning Autoclavable No saccharide derivative leaching High flow rate Useful for very hydrophobic proteins Stable bed volume S: high resolution, high pressure drop, low flow rate C: low resolution, low pressure, high flow rate	HW-40: very effective with organics; phthalates; cyclodextrins; low molecular weight nucleosides/nucleotides; peptide digests HW-50: medium molecular weight molecules; peptides HW-55: medium molecular weight molecules; RNase digest; tissue plasminogen activator; RNA aggregates; glycoproteins HW-65: polysaccharides; mRNA; collagenous proteins HW-75: large molecular weight molecules; amylose subfractions; plasmid DNA; replace ultracentrifugation steps

80 cm water= 0.8 bar = 11.6 psi = 0.08 MPa.

Recommended Reading

Gel Filtration: Principles and Methods (5th Edition)

Cytiva, 1991, 102 pp.

Ever since the introduction of Sephadex™, gel filtration has had a key role in the purification of thousands of biological macromolecules. The enduring value of Sephadex™ depends on the reliability and simplicity of gel filtration as a separation technique. This handbook is a valuable laboratory aid to the selection and use of Cytiva gel filtration media.

Handbook of Size Exclusion Chromatography

Marcel Dekker, 1995, 453 pp.

This book details the practical use of size exclusion chromatography in various application areas.

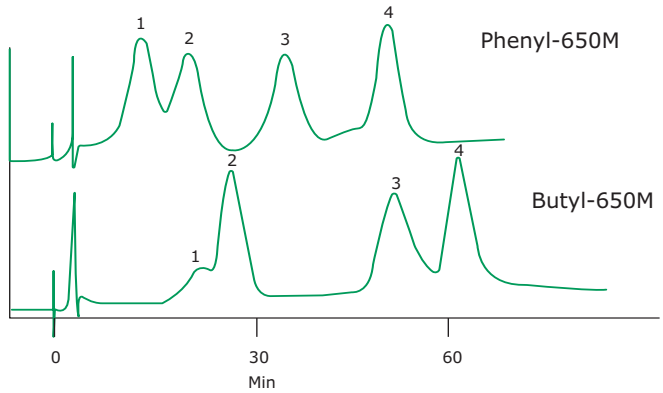
Hydrophobic Interaction Chromatography

Hydrophobic interaction chromatography (HIC) is an alternative to reversed phase chromatography for exploiting the hydrophobic properties of proteins. The addition of a salt to the mobile phase buffer and sample solution promotes protein-medium interactions. The proteins are adsorbed to the medium in a mobile phase containing a high concentration of salt. Most of the bound proteins are effectively desorbed by simply washing with water or a dilute, near-neutral buffer. Because HIC employs a more polar, less denaturing environment than RPLC, it is popular for protein purification, often in combination with ion exchange or gel filtration chromatography.

The commercial availability of well-characterized HIC matrices offers possibilities for purifying a variety of biomolecules, such as serum, membrane, recombinant and nuclear proteins and receptors. The technique is sufficiently sensitive to nonpolar groups normally buried within the tertiary structure of the protein but tend to get exposed if the peptide chain is incorrectly folded or damaged. This sensitivity is useful for separating the native protein from other forms.

The protein adsorption selectivity of HIC medium is primarily determined by the type of immobilized ligand (protein-binding molecule) on the support. In general, alkyl ligands exhibit only hydrophobic character, while aryl ligands exhibit mixed mode behavior - both aromatic and hydrophobic interactions. Very hydrophobic proteins are generally applied to the least hydrophobic media; hydrophilic proteins are purified on the most hydrophobic media. The appropriate HIC medium can reduce salt consumption, and thus lower cost.

The lowest possible salt concentration should be used to bind the protein to the ligand. This trait often depends on the salt chosen. For example, compared to ammonium sulfate or sodium sulfate, up to four times higher concentration of sodium chloride may be needed to obtain the same binding. The salt concentration should be below that which will precipitate the proteins in the crude feed stock. A 1 M solution of ammonium sulfate is most commonly used. A decreasing salt gradient can be used to increase protein resolution.



Hydrophobic Interaction Matrices

Matrix	pH Range	Max. Operating Pressure & Flow	Properties/Limitations
Acrylic Beads 150 µm	cleaning: 0-12 operating: 5-9	0.2 MPa, 100 mL/min (2 x 28 cm column)	Macroporous; stable in organics; hydrophilic, neutral; no shrinking/swelling with changes in ionic strength; mechanically stable; high binding capacity
Agarose, Beaded 2%: 60-200 µm 4%: 45-165 µm 6%: 45-165 µm	4-9	2%: 0.04 MPa, 0.8 mL/min 4%: 0.08 MPa, 1.0 mL/min 6%: 0.20 MPa, 1.2 mL/min (2.5 x 30 cm column)	Analytes up to 4 x 10^7 Da; temperature to 40 °C; sterilizable (chemical); insoluble in all common solvents; should not be used with oxidizing agents or chaotropic salts
Cellulose fibrous tubes, 30-60 µm diam., 85-250 µm long	2-10	0.07 MPa, 2.4 mL/min (2.5 cm column diameter)	Low resolution; pliable material
Sepharose® 2B: 60-200 µm 4B: 45-165 µm 6B: 45-165 µm	4-9	2B: 0.04 MPa, 0.8 mL/min 4B: 0.08 MPa, 1.0 mL/min 6B: 0.20 MPa, 1.2 mL/min (2.5 x 30 cm column)	Analytes up to 4 x 10^7 Da; temperature to 40 °C; sterilizable (chemical); insoluble in all common solvents; should not be used with oxidizing agents or chaotropic salts
Sepharose® CL 2B: 60-200 µm 4B: 45-165 µm 6B: 45-165 µm	3-14	CL-2B: 0.05 MPa, 1.2 mL/min CL-4B: 0.12 MPa, 2.2 mL/min CL-6B: 0.20 MPa, 2.5 mL/min	Analytes up to 4 x 10^7 Da; high flow rates; sterilizable (autoclave/chemical); insoluble in all common solvents; can be used (2.5 x 30 cm column) in dissociating media, high concentrations of chaotropic salts
Toyopearl® 40-90 µm	operating: 2-12 cleaning: 1-13	0.7 MPa	Analytes up to 1 x 10^6 Da; 1000 Å pores; hydrophilic, neutral; compatible with solvents; chemically resistant; volume stable to changes in pH or ionic strength

80 cm water = 0.8 bar = 11.6 psi = 0.08 MPa.

Ion Exchange Chromatography

Ion exchange is the most commonly practiced chromatographic method for purifying proteins and inorganic or organic ions. Compared to other separations, it is widely applicable, easy to scale up, and low in cost. Ion exchange involves solute interactions with the charged groups of the packing material, followed by elution with an aqueous buffer of higher ionic strength or a change in pH.

The Supelco® resins portfolio offers a comprehensive range of ion exchangers, based on various matrices. Well known to the biochemist are agarose-, cellulose-, and dextran-based ion exchangers for low pressure applications. Methacrylate-based ion exchangers are increasingly used in low to medium pressure applications. Our line of polystyrene-based resins includes ion exchangers from DuPont Water Solutions and Mitsubishi Chemical.

Definitions

Gel or microreticular resins do not have true porosity. Instead, solute ions must diffuse through the particle to the ion exchange sites.

Macroreticular resins contain discrete pores which facilitate diffusion of the solute ions to the ion exchange sites. High molecular weight ions that can only sparingly penetrate gel-type resins have easier access to exchange sites in macroreticular beads. The sponge-like structure of macroreticular resins offers superior physical and chemical properties to those available with conventional gel-type resins.

A macroporous resin is a macroreticular resin with more than 20% crosslinkage.

Simple Resin Selection

Product selection charts for each product group (strong anion exchange, weak anion exchange, strong cation exchange, and weak cation exchange) simplify the task of selecting the appropriate resin for your application. In addition, cross-reference charts compare similar (not necessarily equivalent) products from the different manufacturers.

Suggested Regeneration Levels

Exchanger	Ionic Form	Regenerant	Requirement (meq. Regen/meq. Resin)
Strongly Acidic Cation Exchanger	H^+ Na^+	HCl / H_2SO_4 NaCl	3-5 3-5
Weakly Acidic Cation Exchanger	H^+ Na^+	HCl / H_2SO_4 NaOH	1.5-2 1.5-2
Strongly Basic Anion Exchanger Types I & II	OH^- Cl^- SO_4^{2-}	NaOH NaCl/HCl Na_2SO_4/ H_2SO_4	4-5 4-5 4-5
Weakly Basic Anion Exchanger	Free Base Cl^- SO_4^{2}	$NaOH/NH_4OH$/ Na_2CO_3 HCl H_2SO_4	1.5-2 1.5-2 1.5-2 1.5-2

Anion Exchange Media

Matrix	Brand Names/ Functionality	Properties/Limitations	Potential Applications
Agarose DEAE: 45-165 µm Q FF: 45-165 µm Q HP: 24-44 µm	Sepharose® DEAE CL-6B DEAE FF Q FF Q HP	Higher exclusion limits than Sephadex™ Autoclavable FF: extreme chemical & physical stability; high flow rate HP: high-performance CL-6B HP: final polishing; high resolution Clean with 0.1 M sodium hydroxide DEAE CL-6B: low pressure	Q: Higher selectivity than DEAE. proteins; membrane proteins; polysaccharides; nucleic acids; high molecular weight compounds DEAE CL-6B: widely used, well documented FF: initial cleanup; group separations HP: final polishing; high resolution
Cellulose 40-160 µm (microgranular)	DEAE Sephacel™	Low pressure; autoclavable Sephacel™: no precycling or definingpH 2-9 Clean with 0.1 M sodium hydroxide	Proteins; nucleic acids; hormones; other biopolymers Sephacel™: excellent alternative to fibrous forms
Dextran 40-125 µm	Sephadex™ DEAE QAE	High capacity/price ratio Low pressure; autoclavable pH 2-9 Clean with 0.1 M sodium hydroxide A-50: bed volume changes with ionic strength	A-25: low molecular weight proteins; polypeptides; nucleotides. High flow. A-50: batch separation; early steps; radioactive contamination. Lower flow rate. Widely used, well documented.
Methacrylate C: 60-150 µm M: 40-90 µm S: 20-50 µm	Toyopearl® DEAE QAE Q	Stable bed volume High mechanical strength Medium pressure (0.7 MPa) Large pores; high protein recovery pH 2-10 Clean with acid, base, heat	Enzyme/protein purification

Anion Exchange Media (continued)

Matrix	Brand Names/ Functionality	Properties/Limitations	Potential Applications
Polystyrene strong anion most 16-50 mesh (297-1000 µm)	Types I & II Amberlite™ Diaion™	Rigid; low back pressure Thermally & chemically stable; not compatible with strong oxidizers Range of functional groups, moisture content, capacity pH 1-14	Water purification; decolorization; demineralization;neutralization; pharmaceuticals; enzymes; catalysts; sugar refining
Polystyrene weak anion 16-50 mesh (297-1000 µm)	Amberlite™ Diaion™ Alkylamine	Rigid; low back pressure Thermally & chemically stable; not compatible with strong oxidizers Range of moisture content & capacity pH 1-7/9	Water purification; decolorization; demineralization; neutralization; pharmaceuticals; proteins; amino acids; refining

Cation Exchange Media

Matrix	Brand Names/ Functionality	Properties/Limitations	Potential Applications
Agarose CM: 45-165 µm SP FF: 45-165 µm SP HP: 24-44 µm	Sepharose® CM CL-6B FF SP Sepharose® FF HP	Higher exclusion limits than Sephadex™ Autoclavable FF: chemically & physically stable HP: high-performance CL-6B Clean with 0.1 M sodium hydroxide CM: pH 6-10; S: pH 3-11	Proteins; polysaccharides; nucleic acids; membrane components; high molecular weight compounds. Widely used; well documented. FF: initial cleanup; group separations. SP: higher selectivity than CM. HP: final polishing; high resolution
Cellulose Fibrous microgranular	CM Phosphate	Microgranular: better flow & pressure stability	CM: highly basic materials
Dextran 40-120 µm	Sephadex™ CM SP	High capacity Low pressure; autoclavable Clean with 0.1 M sodium hydroxide CM: pH 6-10; SP: pH 2-10 C-50: bed volume changes with ionic strength	C-25: low molecular weight proteins; polypeptides; nucleotides. High flow. C-50: batch separations; crude samples; early steps; radioactive contamination. Lower flow rate. Widely used, well documented
Methacrylate C: 60-150 µm M: 40-90 µm S: 20-50 µm	Toyopearl® CM SP	Stable bed volume High mechanical strength Medium pressure (0.7 MPa) Large pores; high protein recovery Clean with acid, base, or heat CM: pH 5-10; SP: pH 3-11	Enzyme/protein purification
Polystyrene strong cation most 16-50 mesh (297-1000 µm)	Amberlite™ Diaion™	Rigid Thermally & chemically stable; not compatible with strong oxidizers Range of particle sizes, moisture content, capacity pH 1-14; 120 °C/150 °C maximum	Water conditioning; neutralization; amines; metals; pharmaceuticals; amino acids; catalysts; refining; deionization
Polyacrylic weak cation most 16-50 mesh (297-1000 µm)	Amberlite™ Diaion™	Rigid; low back pressure Thermally & chemically stable; not compatible with strong oxidizers Range of moisture content and capacity pH 4/5; 120 °C maximum	Water conditioning; neutralization; amines; metals; proteins; amino acids; pharmaceuticals; peptides; deionization

Ion Exchange Media

Comparable Styrene/DVB Ion Exchange Resins

These cross-reference charts compare similar (not necessarily equivalent) products from major ion-exchange resin manufacturers. For characteristics of each resin, consult **SigmaAldrich.com**. These charts do not list all available resins, but just the comparable ones.

Comparable Anion Exchangers on Polystyrene

	Amberlite™	Amberchrom™ / AmberSep™ / AmberTec™ / Amberlyst™
Strong Type I	IRA-900 MSA-1 IRA-958 IRA-402 Amberjet® 4200 IR-120 HPR4200 HPR9200 HPR1200 HPR4811 HPR4800	1X4 11/21K/SBR/SBR-P A26 900 1X2 1X8 550/A/G-55/N-196, XUS -40196.01 SBR-C
Strong Type II	IRA-910 HPR4100 FPA22	
Weak	IRA-67 IRA-93 IRA-96 HPR4100 HPR4800 FPA66	WA30

Comparable Strongly Acidic Cation Exchangers on Polystyrene

Amberlite™	Diaion™	Amberchrom™ / Amberlyst™
IRC-120 Plus (H) FPC23 HPR1100 HPR2900 HPR650	PK228	50WX4 50WX2 50WX8 G-26 N-437 36 15

Comparable Weakly Acidic Cation Exchangers on Polyacrylic Copolymer

Amberlite™	Diaion™
CG-50 Type I IRC-76 MAC-3	PK228

Comparable Chelating Resins

Diaion™
CR11

Comparable Mixed Bed Resins on Polystyrene

Amberlite™
MB-1 MB20 MB-9L MB-6113

Comparable Nuclear Resins

Amberlite™
IRN-78 IRN-150 MB-20

In addition to these resins we also have special ion exchange resins like neutral ion exchangers (Amberlyst™ A21), strong basic cation exchange (HPR650H), and weakly basic anion exchange (IRA-743).

Contact our Technical Service Department
for expert answers to your questions.

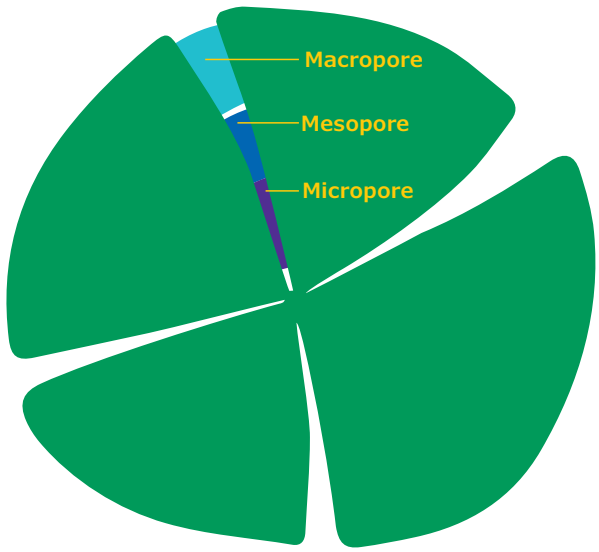
Carboxen® Synthetic Carbon

Activated carbons are widely used for purification and separation applications. However, the use of traditional, naturally derived activated carbons is hindered due to chemical purity, irregular shape, mechanical integrity, and lack of reproducibility. Carboxen® synthetic carbon adsorbents are a synthetic form of activated carbon. These adsorbents can be thought of as derivatized resins. Due to their high purity, spherical shape, and outstanding mechanical properties, Carboxen® synthetic carbon adsorbents are well suited for use in LPLC applications. This packing media can withstand pressures up to 110 MPa which is several orders of magnitude greater than other LPLC media. The tapered pores improve the adsorbent's kinetic and thermodynamic efficiency, enabling greater capacity while operating at increased throughputs.

Carboxen® Synthetic Carbon Material Properties

	Surface Area (m^2/g)	Surface pH (ASTM D6851)	1 = most hydrophilic 10 = most hydrophobic	Pore Structure (%)			Ball Pan Hardness (ASTM D3802)
				Micro (< 20 Å)	Meso (20 - 500 Å)	Macro (>500 Å)	
Carboxen® 563	500	6.8	7	38	24	38	99.8
Carboxen® 564	400	8.7	8	47	26	27	99.8
Carboxen® 569	500	8.6	9	45	32	23	99.4
Carboxen® 572	1000	9.5	4	48	23	29	99.1
Carboxen® 1005	1000	8.0	10	47	26	27	99.6
Carboxen® 1032	800	3.0	1	49	0	51	98.6
Carboxen® 1033	400	7.0	3	38	0	62	99.7
Carboxen® 1034	1200	10.5	2	52	3	45	98.9

The highly engineered, derivatized resins bring value in both adsorption and hydrophobic interaction chromatography. The media are offered in a range of particle sizes with 20-40 mesh being the standard particle size range. These adsorbents are stable over the entire pH range and can often be regenerated.



Functionalization of carbon surfaces with oxygen functional groups alters the materials' pH and hydrophobic/hydrophilic properties. The pH can be tailored from 2.5 to 10.5 and selected to meet the application needs. A graphene-like surface free of functionality provides a very hydrophobic media as is the case for Carboxen® 569 and 1005 synthetic carbon. These media bring value to a wide range of LPLC applications for both small and large molecule separations. As example, these adsorbents are used for clearance of high-risk impurities in biochemical purifications such as host cell proteins, trace metals, and extractables and leachables. These synthetic carbons have been trusted by NASA on several missions including their use on the International Space Station.

Supelco®

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