

Supelco LC Media Selection Guide

Low pressure liquid chromatography (LPLC) is the oldest of chromatographic techniques, originally performed in open columns in which gravity moved the sample through the packing bed. Recently, pumps and other instrumentation have been incorporated into LPLC and superior packing media have been developed specifically for this versatile technology. The Supelco Media Store has most of the best media, and packed columns, empty columns, filters, injectors, valves, and tubing for LPLC. The media in this bulletin are described alphabetically by chromatography mode: adsorption, affinity, gel filtration, hydrophobic interaction, and ion exchange. Empty columns and other hardware are listed in the Supelco catalog.

Key Words:

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

Low pressure liquid chromatography (LPLC), the oldest of chromatographic techniques, formerly was simply called liquid chromatography. 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 endfitting 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 added via a pipette, the solvent is carefully added without disturbing the sample band, and the solvent reservoir is reattached.

In recent years, 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 has become 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 chromatography mode: adsorption, affinity, gel filtration, hydrophobic interaction, and ion exchange. Media for reversed phase LPLC are included with the adsorption media.

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, which depends on the LPLC application. Traditional inorganic adsorbents include silica and bonded silica, alumina, carbon, and Florisil®. The most important use for inorganic matrices is in the purification and isolation of small molecular weight compounds, including environmental analytes. Polymeric adsorbents have important applications in the pharmaceutical industry for, e.g., purification of antibiotics from fermentation broths. Their 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 many new media as well as new LC techniques. Among the first media biochemists used to purify proteins were dextran-based Sephadex® beads for gel filtration and ion exchange. 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. Recent developments include media that have better pressure stability and resolution (e.g., Toyopearl® and Superdex® media) to allow higher flow rates and reduced purification times.

Column Hardware, LPLC Accessories

Glass and inert plastic are the most common column materials. Depending on what solvents will 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®.

Empty columns of various design, fittings, injectors, valves, filters, and tubing suitable for low pressure liquid chromatography are listed in the Supelco catalog.

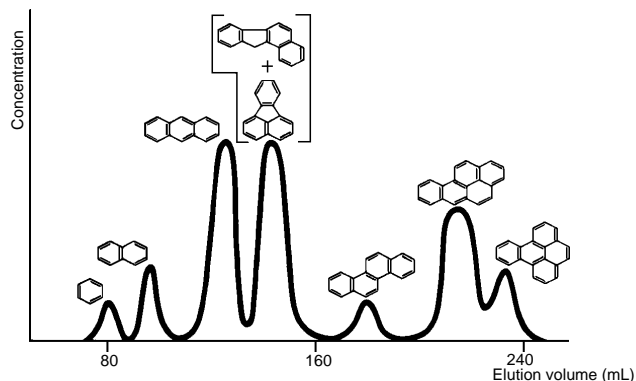
Adsorption Chromatography

Adsorption and partition chromatography are used in a wide variety of synthetic and biochemical separations. Commonly, 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 in alphabetical order by type in the Supelco catalog.

PAHs on Sephadex LH-20

Packing: **Sephadex LH-20**
 Cat. No.: **LH-20-100B** (50g)
 Column: 1.1 x 112cm
 Mobile Phase: isopropanol
 Flow Rate: 2.5mL/hr
 Inj.: 0.25mL (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, Ambersorb®	Toxic emissions in air; ultrapure water preparations; liquid/vapor phase separations
Dextran, Hydrophobic 10-23µm, 25-100µm	Bulk packing, hydroxyalkoxypropyl dextran, Sephadex LH-20	Varying hydrophobicities. Gel permeation chromatography; lipids; steroids; fatty acids; vitamins
Florisil (Magnesium Silicate) <74-1200µm 150-250µm (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 100µm	Bulk packing, ORBO tubes, Solid phase extraction tubes BioPure™ cartridges Amberlite XAD®, Amberchrom®, Diaion®, DOWEX®, Supelite™ DAX™-8, Supelpak™	Surfactants; pharmaceuticals; environmental analysis; organics; wastewater treatment; pesticides; humic substances; vitamins; decolorization
Silica purified: 15-650µm, 20-150Å bonded RP phases: 20-63µm, 60Å, 300Å	Bulk packing Davisil®, E. Merck, Hypersil®, Sigma-Aldrich™, SPELCO SIL™ A	Flash chromatography; cleanup and purification of a wide range of synthetic and natural compounds

Affinity Chromatography

Affinity Media

The Supelco Media Store offers a large variety of affinity media, in bulk or in prepacked columns. For affinity media and Toyopearl resins, HiTrap® affinity columns (from Pharmacia Biotech) and prepacked columns, request publication 996002.

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 matrix is stable at pH 2-12, but the specific ligand chemistry may limit the usable range. Toyopearl Epoxy media are used at pH 9-11, 40°C to make a stable secondary amine linkage, but at pH 7-8, 25°C for a stable sulfide linkage.

Affinity Matrices

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

80cm H₂O = 0.8 bar = 11.6psi = 0.08MPa.

Affinity Ligands

The ligand should exhibit specific and reversible binding affinity for the substance to be purified. In addition, it should have chemically modifiable groups that allow it to be attached to the matrix without destroying its ligand-binding activity. The dissociation constant (K_d) for the ligand-binding substance complex should ideally be in the range of 10⁻⁴ to 10⁻⁸M 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 the specific interaction with the molecule to be isolated.

Spacer Arms

The active site on a biological molecule often is 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, 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

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 to which proteins, nucleic acids, or other biopolymers then can be coupled, under mild conditions, via primary amino or similar nucleophilic groups.

Resins with *reactive groups* employ carbodiimide coupling or reductive amination to achieve covalent bonding. For example, the Toyopearl AF-Amino-650M matrix can be used to couple ligands via their carboxylate groups (peptide bond formation) or aldehyde groups (reductive amination). 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 attachment to activated matrix is made

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 non-denaturing 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 MW 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

Pharmacia Biotech, 1993, 143 pp.

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

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For a detailed description of our affinity line, ask for publication 996002.

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 given to immunochemical techniques, including antibody isolation, preparation of antibody fragments using immobilized enzymes, and immunoaffinity chromatography. Describes processes for making optimized affinity supports.

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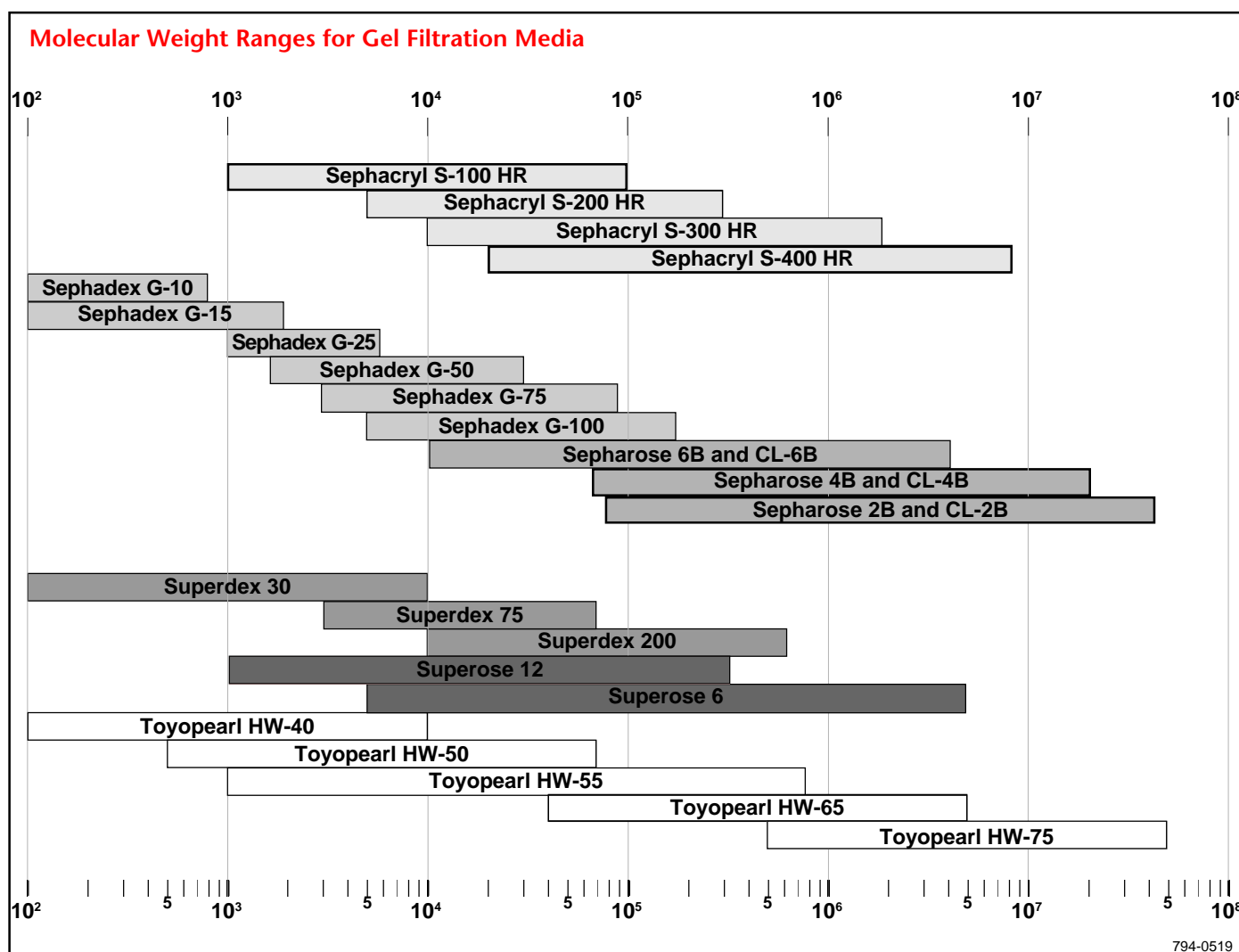
Gel Filtration Chromatography

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 which analytes are fully excluded from entering the pores and thus will not be separated. According to their size, smaller analytes have partial to complete access to the pore volume. Among the analytes which partially or fully enter the pore volume, larger molecules with less 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 Media Store 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 Pharmacia Biotech AB and Tosoh Corporation.

Media selection charts in this bulletin contain pertinent information about each gel filtration packing material. Organized alphabetically by media name, 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 Supelco catalog for Sephadex LH-20, which is used for purifying small molecular weight compounds by gel permeation and partition chromatography. Hydroxyalkoxypropyl dextran also are listed with the adsorption media.



Gel Filtration Chromatography

Gel Filtration Media

Medium	Fractionation Ranges (proteins)	Properties/Limitations	Potential Applications
Sephacryl			
Matrix: acrylic (dextran/bisacrylamide copolymer)			
25-75µm	S-100 HR: 1K-100K	Clean with 0.2M NaOH, 0.1M HCl, 1.0M HOAc	S-100 HR: peptides, small proteins
S-1000: 40-105µm	S-200 HR: 5K-250K	Compatible with detergents, chaotropic salts, dissociating agents Sterilize by autoclave Compatible with organic solvents Scaleable Good recoveries Stable bed volume	S-200 HR: proteins, some small serum proteins (e.g., albumin)
0.2MPa	S-300 HR: 10K-1500K		S-300 HR: membrane & serum proteins, monoclonal antibodies
(2.6 x 30cm column)	S-400 HR: 20K-8000K		S-400 HR: large proteins & macromolecules with extended structures (e.g., proteoglycans, liposomes)
pH range: 2-11	S-500 HR, S-1000: ND		S-500 HR: large macromolecules, small particles (e.g., plasmids)
Sephadex			
Matrix: dextran (crosslinked with epichlorohydrin)			
C: 100-300µm	G-10: < 700	Classic support	G-10: very low MW substances; desalting; buffer exchange;
M: 50-150µm	G-15: < 1500	Swelling 2-40X	peptides
F: 20-80µm	G-25: 1K-5K	Use LH grades for organic solvents	G-15: low MW compounds; interact with aromatic compounds;
SF: 10-40µm	G-50: 1.5K-30K	SF grade for best resolution, low flow rate	desalting; buffer exchange; peptides
SF: 0.016-0.160MPa	G-75: 3K-80K	F grade for prep lab work	G-25: medium grade available in packed PD-10 columns for rapid, routine desalting;
(2.6 x 30cm column)	G-100: 4K-150K	M grade for standard lab work	interact with aromatic compounds; small peptides & proteins; buffer exchange
pH range:		C grade for very crude samples	G-50: desalting; buffer exchange; standard for process scale; very low nonspecific interactions
2-13 (< G-25)		Can be autoclaved at 120°C	G-100: very low nonspecific interactions
2-10 (> G-25)		Compatible with detergents, chaotropic salts, dissociating agents	
		Clean with 0.2M NaOH	
		Ideal for MW determinations	
		Interact with aromatic compounds	
Sepharose			
Matrix: agarose			
6B: 45-165µm	6B: 10K-4000K	Clean with 0.5M NaOH	2B: DNA-protein complexes; viruses;
4B: 45-165µm	4B: 60K-20,000K	Good recoveries	asymmetric molecules; affinity support
2B: 60-200µm	2B: 70K-40,000K	Broad fractionation range	4B: tRNAs; membrane proteins;
0.004-0.02MPa		Temperature range: 4-40°C	polysaccharides; affinity support
(2.6 x 30cm column)		Cannot be autoclaved	6B: polio virus purification; proteins;
pH range: 4-9		Incompatible with oxidizing agents, chaotropic salts, dissociating agents	polysaccharides; affinity support
Sepharose CL			
Matrix: agarose (Sephacryl reacted with 2,3-dibromopropanol)			
6B: 45-165µm	6B: 10K-4000K	Stable in chaotropic salts, dissociating agents	2B: very high MW molecules (e.g., nucleic acids); polysaccharides;
4B: 45-165µm	4B: 60K-20,000K	Flow 2X that of Sepharose	cell particles; viruses
2B: 60-200µm	2B: 70K-40,000K	Sterilize by autoclave	4B: vesicles; membrane proteins; poly- saccharides; affinity support
0.005-0.02MPa		Easy switch to organic solvents	6B: viruses; proteins; polysaccharides;
(2.6 x 30cm column)		Good recoveries	affinity support.
pH range: 3-14		Broad fractionation range	
		Ideal for long-term routine applications	
		Do not use with oxidizing agents	

80cm H₂O = 0.8bar = 11.6psi = 0.08MPa

Gel Filtration Chromatography

Gel Filtration Media (contd.)

Medium	Fractionation Ranges (proteins)	Properties/Limitations	Potential Applications
Superdex			
Matrix: dextran (covalently bonded to agarose) 24-44µm 0.5MPa pH range: 3-12	30: ≤10K 75: 3K-70K 200: 10K-600K	Newest member of GFC family Clean with 1M NaOH, 0.1M HCl, 1.0M HOAc Can be used with detergents, chaotropic salts, dissociating agents Sterilize by autoclave 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 MW (albumin, transferrin); good when protein MW is unknown. Selectivity similar to Sephadex G-200
Superose			
Matrix: agarose (highly crosslinked) 20-40µm 12: 0.7MPa 6: 0.4MPa (1.6 x 50cm column) pH range: 2-14	12: 1K-300K 6: 5K-5000K	Clean with 0.2M NaOH, 0.01M HCl, 1.0M HOAc 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: 50-100µm F: 30-60µm S: 20-40µm 0.7MPa 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 MW nucleosides/nucleotides; peptide digests HW-50: medium MW molecules; peptides HW-55: medium MW molecules; RNase digest; tissue plasminogen activator; RNA aggregates; glycoproteins HW-65: polysaccharides; mRNA; collagenous proteins HW-75: large MW molecules; amylose subfractions; plasmid DNA; replace ultracentrifugation steps

Recommended Reading

Gel Filtration: Principles and Methods (5th Edition)

Pharmacia Biotech, 1991, 102 pp.

Since the introduction of Sephadex, thirty years ago, 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 Pharmacia Biotech gel filtration media.

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Handbook of Size Exclusion Chromatography

Marcel Dekker, 1995, 453 pp.

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

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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 becoming popular for protein purification, often in combination with ion exchange or gel filtration chromatography.

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

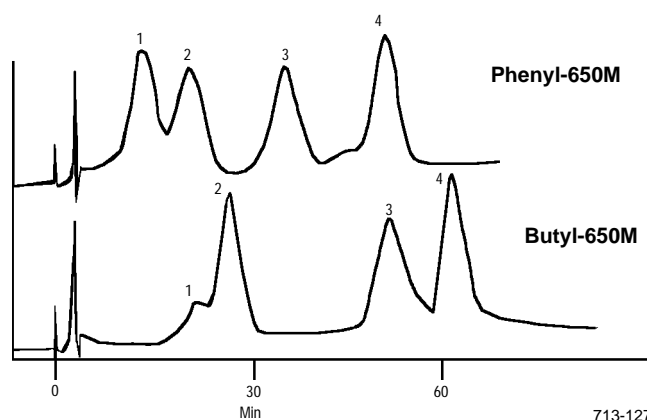
The protein adsorption selectivity of an HIC medium is primarily determined by the type of immobilized ligand (protein-binding molecule) on the support. In general, alkyl ligands exhibit only hydrophobic character, while aryl ligands exhibit mixed mode behavior – both aromatic and hydrophobic interactions are possible. 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 often depends on the salt chosen. For

example, compared to ammonium sulfate or sodium sulfate, an 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 1M solution of ammonium sulfate is most commonly used. A decreasing salt gradient can be used to increase protein resolution.

Selectivity of Toyopearl HIC Resins

Packing: **Toyopearl-Phenyl 650M**
Toyopearl-Butyl 650M
 Cat. No.: **814478 (Phenyl)**
807477 (Butyl)
 Column: 7.5cm x 7.7mm ID
 Mobile Phase: 1.8M (NH₄)₂SO₄ in 0.1M phosphate buffer, pH 7 (A),
 0.1M phosphate buffer, pH 7 (B)
 from 100% A to 100% B in 60 min
 Flow Rate: 1.0mL/min
 Det.: UV, 280nm
 Inj.: 1. myoglobin, 2. ribonuclease A, 3. lysozyme,
 4. α-chymotrypsinogen



Hydrophobic Interaction Matrices

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

80cm H₂O = 0.8 bar = 11.6psi = 0.08MPa.

Ion Exchange Chromatography

Ion exchange is the most commonly practiced chromatographic method for purifying proteins and inorganic or organic ions. Relative 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 Media Store 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 being used in low to medium pressure applications. Our line of polystyrene-based resins includes ion exchangers from Rohm and Haas Co., The Dow Chemical Co., 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 which 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.

Anion Exchange Media

Matrix	Brand Names/ Functionality	Properties/Limitations	Potential Applications
Agarose DEAE: 45-165µm Q FF: 45-165µm Q HP: 24-44µm	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 Clean with 0.1M NaOH DEAE CL-6B: low pressure	Q: Higher selectivity than DEAE. proteins; membrane proteins; polysaccharides; nucleic acids; high MW compounds DEAE CL-6B: widely used, well documented FF: initial cleanup; group separations HP: final polishing; high resolution
Cellulose 40-160µm (microgranular)	DEAE Sephacel	Low pressure; autoclavable Sephacel: no precycling or defining pH 2-9 Clean with 0.1M NaOH	Proteins; nucleic acids; hormones; other biopolymers Sephacel: excellent alternative to fibrous forms
Dextran 40-125µm	Sephadex DEAE QAE	High capacity/price ratio Low pressure; autoclavable pH 2-9 Clean with 0.1M NaOH A-50: bed volume changes with ionic strength	A-25: low MW proteins; polypeptides; nucleotides. High flow. A-50: batch separation; early steps; radioactive contamination. Lower flow rate. Widely used, well documented.
Methacrylate C: 60-150µm M: 40-90µm S: 20-50µm	Toyopearl DEAE QAE Q	Stable bed volume High mechanical strength Medium pressure (0.7MPa) Large pores; high protein recovery pH 2-10 Clean with acid, base, heat	Enzyme/protein purification
Polystyrene strong anion most 16-50 mesh (297-1000µm)	Types I & II Amberlite Diaion DOWEX	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

Simple Resin Selection

Product selection charts for each product group (strong anion exchange, weak anion exchange, strong cation exchange, 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

Exchange Type	Ionic Form	Regenerant	Requirement (meq. Regen/ meq. Resin)
Strongly Acidic Cation Exchanger	H ⁺ Na ⁺	HCl / H ₂ SO ₄ NaCl	3-5 3-5
Weakly Acidic Cation Exchanger	H ⁺ Na ⁺	HCl / H ₂ SO ₄ NaOH	1.5-2 1.5-2
Strongly Basic Anion Exchanger Types I & II	OH ⁻ Cl ⁻	NaOH NaCl / HCl	4-5 4-5
Weakly Basic Anion Exchanger	Free Base Cl ⁻ SO ₄ ⁻²	NaOH / NH ₄ OH/ Na ₂ CO ₃ HCl H ₂ SO ₄	1.5-2 1.5-2 1.5-2

Ion Exchange Chromatography

Anion Exchange Media (contd.)

Matrix	Brand Names/ Functionality	Properties/Limitations	Potential Applications
Polystyrene weak anion 16-50 mesh (297-1000µm)	Amberlite Diaion alkylamine DOWEX polyamine Duolite® polyamine	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: 45-165µm SP FF: 45-165µm SP HP: 24-44µm	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 0.1M NaOH CM: pH 6-10; S: pH 3-11	Proteins; polysaccharides; nucleic acids; membrane components; high MW 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 40-120µm	Sephadex CM SP	High capacity Low pressure; autoclavable Clean with 0.1M NaOH CM: pH 6-10; SP: pH 2-10 C-50: bed volume changes with ionic strength	C-25: low MW proteins; polypeptides; nucleotides. High flow. C-50: batch separations; crude samples; early steps; radioactive contamination. Lower flow rate. Widely used, well documented.
Methacrylate C: 60-150µm M: 40-90µm S: 20-50µm	Toyopearl CM SP	Stable bed volume High mechanical strength Medium pressure (0.7MPa) 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 (297-1000µm)	Amberlite Diaion DOWEX Duolite SO ₃ H	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 (297-1000µm)	Amberlite Diaion DOWEX Duolite COOH	Rigid; low back pressure Thermally & chemically stable; not compatible with strong oxidizers Range of moisture content and capacity pH 4/5-14; 120°C maximum	Water conditioning; neutralization; amines; metals; proteins; amino acids; pharmaceuticals; peptides; deionization

Through our Cleaned-Certified-Packaged Media program, we can provide sterile, ready-to-use cartridges containing any of the LC media in our catalog. For more information about this program, see our current catalog.

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 your current Supelco catalog. These charts do not list all available resins, just the comparable ones.

Comparable Anion Exchangers on Polystyrene

	Amberlite	Diaion	DOWEX	Duolite
Strong Type I	IRA-900	PA308/PA312	11/MSA-1	A-161 [■] /165 [■]
	IRA-904 [■]	PA308	11/MSA-1	A-171 [■]
	IRA-938 [■]			ES-181 [■]
	IRA-958			A-173 [■]
	IRA-400	SA10A	SBR	A-101 [■] /104 [■] /113 [■]
	IRA-400(OH)	SA10A	SBR	A-101 [■] /113 [■]
	IRA-401S [■]	SA11A	1X4	A-147 [■]
	IRA-402	SA12A [■]	11/SBR-P	A-113 [■]
	IRA-420C [■]	SA10A	11/21K/SBR/SBR-P	A-113 [■]
	IRA-458			A-132 [■]
		HPA25		
		PA306S		
	IRA-420C [■]	NSA100		
		SA10AS		
	Amberjet [®] 4200		1X2 1X8 550/A/G-55/N-196, XUS -40196.01 SBR-C SBR-P-C	
Strong Type II	IRA-910	PA418	MSA-2	A-162 [■]
	IRA-410/416 [■]	SA20A	SAR	A-102 [■] /116 [■]
		HPA75		
		PA408		
		SA21A		
	WA21J			
			2X8 A2 N-189	
Weak	IRA-35 [■]			
	IRA-67	WA103 [■]		A-375 [■]
	IRA-92			
	IRA-93, IRA-95	WA30	66/MWA-1	A-378 [■]
	IRA-94 [■] , IRA-96	WA30	N-283	A-378 [■]
		WA11 [■]		
		WGR-2 WBA*		A-7 A-368 [■]

[■]Not available from Supelco. *Marathon exchanger

Comparable Strongly Acidic Cation Exchangers on Polystyrene

Amberlite	Diaion	DOWEX	Duolite
200/252 [■]	PK228/PK216 [■]		C-26 [■] /C-265 [■]
IR-118H [■] , 31 [■]			C-206 [■]
IR-120 Plus (H)	SK1B	HCR-S	C-20 [■]
IR-122	SK110 [■]	HGR	C-255 [■]
	SK104	50WX4	C-206 [■]
	PK208		
	HPK25		
IR-124 [■]	SK112	50WX12 [■]	C-255 [■]
	SK1BS		
	SK116	50WX16 [■]	
		50WX2	
Amberjet 1200		50WX8	
		G-26/650C	
		N-437/N-406	
252 [■]			C-280
			C-291

[■] Not available from Supelco.

Comparable Weakly Acidic Cation Exchangers on Polyacrylic Copolymer

Amberlite	Diaion	DOWEX	Duolite
CG-50 Type I	WT01S		
CG-50 Type II			
DP-1	WK100(Na) [■]		C-476 [■]
IRC-50	WK100		C-462 [■]
IRC-76/84 [■]	WK20 [■] , WK40	CCR-2 [■] /MWC-1 [■]	C-476 [■]
IRC-84 [■] /86	WK20 [■] , WK40	CCR-3 [■] /MAC-3	C-433 [■]
IRC-50			C-464 [■]

[■]Not available from Supelco.

Comparable Chelating Resins

Amberlite	Diaion	Duolite
IRC-718	CR10 [■]	
	CR11	C-467
	CR20	
		GT-73

[■]Not available from Supelco.

Comparable Mixed Bed Resins on Polystyrene

Amberlite	Diaion	DOWEX	Duolite	Other
MB-1, MB-150	SMNUP	MR-3 11A8 Retardation MR-3C MR-12	MB5113 [■]	
MB-3 [■]	SMT100		MB6113 [■]	TMD-8

[■]Not available from Supelco.

Comparable Nuclear Resins

Amberlite	Diaion	DOWEX
IRN-77	SAN 1 [■]	
IRN-78	SKN 1 [■]	
IRN-150	SMN 1 [■] , SMNUP	MR-3, MR-3C

[■]Not available from Supelco.

Literature Applications for Amberlite/Duolite Cation Exchange Resins

A survey of the literature from 1987 to 1991. **T412140**

Literature Applications for Amberlite/Duolite Anion Exchange Resins

A survey of the literature from 1987 to 1991. **T412141**

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