



## Watch

In this month's "Column Watch," the author focuses on some of the latest developments in preparative-scale columns, bulk packing materials, and column hardware designs. Silica, polymeric, and other packings are discussed along with the newest monolithic columns, which can offer advantages over conventional packed columns. The techniques of flash — and supercritical fluid — preparative chromatography are discussed briefly.

**Ronald E. Majors**  
Column Watch Editor

# Developments in Preparative-Scale Chromatography Columns and Accessories

The definition of preparative liquid chromatography (LC) has always been fuzzy and is dependent upon the eyes of the beholder because the mass of the injected or collected sample often is dependent upon the amount available or the intended need. For some, tens of nanograms is sufficient for compound identification, for others a few micrograms of material is enough for further characterization, while for still others, tens of grams represents a preparative amount.

Recently, I examined the role of the column in preparative chromatography (1). In this paper, I will discuss recent innovations in preparative columns and bulk packings that have caused a renewed interest in this technique, as was noted in my recent coverage of new products for Pittcon 2004 (2). Also, I have noted some highlights of preparative papers presented at Pittcon 2004 and at HPLC 2004.

### Column Parameters of Importance in Preparative Chromatography

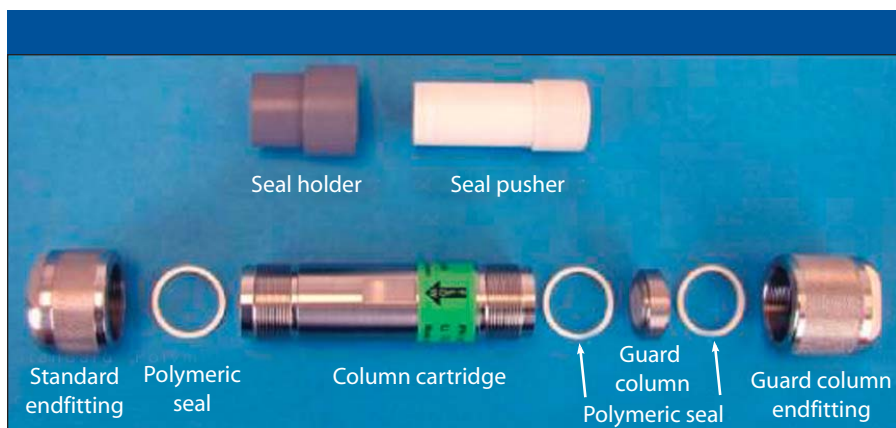
Table I gives a brief summary of the important properties of packing materials used in preparative separations. Many of these parameters also are important in analytical high performance liquid chromatography (HPLC), but some are of even more importance when one scales up to larger column sizes. An ideal preparative column would excel in all of these attributes, but because chromatography is always a compromise, tradeoffs often are required.

### New Conventional Column Products for Preparative Chromatography

**Silica-based packings and columns:** The most widely used packing materials in preparative chromatography are the silica-based particles. Although irregular particles still are available for preparative columns, most of the more recent introductions are based upon spherical packings. Spherical silica particles are stronger and are packed

more easily and reproducibly. Most introductions in this field are from companies already in the analytical columns market, so scalability rarely is an issue. At this year's Pittcon in Chicago, Illinois, both Agilent Technologies (Wilmington, Delaware) and Waters (Milford, Massachusetts) introduced new product lines in conventional silica gel-based preparative columns. The Agilent preparative offering is based upon 100-Å spherical Type B silica (450 m<sup>2</sup>/g surface area, 5- and 10-µm particle diameters) with a new cost-effective, high loadability (milligram to gram quantities) base material to complement the company's existing Zorbax PrepHT line. The columns are available with 21.2-, 30-, and 50-mm inner diameters and 50–250 mm lengths in a cartridge form with an integrated guard column and finger-tight connections. Underivatized silica and C18 reversed-phase chemistries are available in packed columns and in bulk quantities. A matching set of analytical (scalar) columns is available for linear scaleup. Waters' preparative columns incorporate a new optimum bed density design that provides improved efficiency, stability, and life while maintaining the integrity of a separation developed on the previous format. These columns are available with the XTerra hybrid and Atlantis packing materials. These families of packings offer a wide variety of available chemistries, particle sizes, and column inner diameters to allow easier and faster method development, method transfer, and scale up to preparative chromatography.

There is a great deal of interest in the separation of chiral compounds in the pharmaceutical industry. He and colleagues (3) have prepared chiral stationary phases by bonding the macrocyclic glycopeptides vancomycin, teicoplanin, teicoplanin aglycone, and ristocetin A to silica gel. The complex structures of these phases allow them to interact with chiral molecules through many different kinds of forces including ionic (electrostatic) interaction,



**Figure 1:** Zorbax Prep HT and Agilent Prep Cartridge column hardware with integrated guard column. Picture shows schematic of column, guard column, and reusable endfittings with seals, along with seal insertion tools. (Courtesy of Agilent Technologies.)

hydrogen bonding,  $\pi$ - $\pi$  interaction, inclusion complexation, hydrophobic interaction, and steric (repulsion) hindrance.

#### Polymer-based and other materials:

Aside from silica, columns based upon other spherical packings also have appeared on the market. Organic polymers based upon poly(styrene-divinylbenzene) (PS-DVB) have been among the more prominent. An example would be Grace Vydac (Hesperia, California), which developed a 300-Å pore size PS-DVB that was introduced three years ago. The polymeric materials with particles diameters of 8 and 15  $\mu\text{m}$  were developed with protein and peptide separations in mind. The columns can be used in or cleaned with caustic solution, where a silica gel-based material often has shortcomings. Recently, Polymer Laboratories (Amherst, Massachusetts) introduced its own PS-DVB protein material that was deemed to be as mechanically stable as silica gel packing. The company has have derivatized the same production material to produce strong anion and strong cation exchangers. The PS-DVB production process is the subject of a Type II Drug Master File with a single lot size of 200 kg, equivalent to 600 L of HPLC column packed bed.

In addition to polymeric monoliths, covered next, new specialty polymers have been developed for specific applications. Amersham Biosciences (Piscataway, New Jersey) recently has introduced its Glutathione Sepharose High Performance, which consists of 6% highly cross-linked 34- $\mu\text{m}$  agarose beads to which glutathione has been immobilized. The resulting medium selectivity retains GST-tagged recombinant proteins from bacterial lysates and other S-transferases or glutathione-

dependent proteins, allowing them to be purified from cellular contaminants in a one-step purification. The GST-tagged proteins are eluted under mild, nondenaturing conditions using reduced glutathione. The purification process preserves protein antigenicity and function.

Monoclonal antibodies can be used therapeutically to protect against disease; they also can help to diagnose a wide variety of illnesses and detect the presence of drugs, viral and bacterial products, and other unusual or abnormal substances in the blood. The purification of monoclonal antibodies was addressed by Zirchrom (Anoka, Minnesota), which developed a novel stationary phase based upon porous zirconia particles chemically modified with *N,N,N',N'*-ethylene-diaminetetra-(methylphosphonic) acid. This phase, which is a pseudo-affinity stationary phase because it is not an immobilized antigen, is useful for small and large-scale purification of biomolecules. Compared with soft gels, the rigid material can be run at very high mobile-phase linear velocities that result in increased sample throughput. A maximum dynamic bead capacity was measured for polyclonal hIgG to be 20 mg/mL at a linear velocity of 3 cm/min. The product was compared to Protein G media and found to provide a 100-fold increase in purification speed with an improved monoclonal antibody purity and recovery as measured by enzyme-linked immunosorbent assay (ELISA) (4).

Shiseido (Tokyo, Japan) has developed a novel chiral stationary phase that utilizes spherical clay as the support material and a ruthenium complex as the chiral selector (5). This chiral stationary phase was characterized by high sample loadability, compati-

bility with most organic solvents, a wide range of temperature, and overall robustness.

#### Preparative Monolithic Columns

Preparative monoliths seem to be getting center stage as the newest innovation. A positive feature of monoliths is their high permeability so that for preparative work, they can operate at a high flow rate and still exhibit good efficiency. In addition, their sample capacity also holds up under high velocity conditions. At Pittcon 2004, both polymeric and silica-based monolith preparative columns were introduced. One of the early leaders in polymer-based monolith columns, BIA Separations (Ljubiana, Slovenia), expanded its line of methacrylate copolymer convective interaction media (CIM) columns to include a current good manufacturing practice (cGMP) 800-mL Tube Monolithic column.

**A positive feature of monoliths is their high permeability so that for preparative work, they can operate at a high flow rate and still exhibit good efficiency.**

This column, based upon a poly(glycidyl-methacrylate-co-ethyleneglycoldimethacrylate) polymer, was functionalized with a diethylamino group to be used for anion-exchange separations. With a dynamic protein-binding capacity of 20–60 g protein/mL wet support and as much as 25 mg of TmoV virus/mL of wet support, this column is focused on industrial scale biochromatography and is the first cGMP-compliant, industrial-scale monolith with a Drug Master File and other documentation for scale up from research purification. With a 65-mm inner diameter and a 15-cm length, it can handle flow rates as high as 2 L/min with a backpressure below 5 bar. An 8-L column is available for even larger sample masses. The company also has displayed a monolith with epoxy functional groups that can be used to immobilize antigens or other reactive compounds. With comparable analytical columns available, biochromatographers now have the ability

**Table 1: Important properties of preparative packing materials**

Column/Packing Property	Reason for Importance	Relative Importance
Surface Properties		
Retention	Based upon phase chemistry; determines separation time and volume of solvent required (related to expense); dependent upon surface area, ion-exchange capacity or bonded-phase loading; in reversed-phase hydrophobicity, also will be affected by type of ligand (C18 vs. C3 vs. cyanopropyl)	Moderate
Selectivity	Based upon phase chemistry and mobile-phase conditions; affects analyte purity when column is run in an overloaded condition; mixed mechanisms can affect selectivity (for example, ion-exchange and hydrophobic interactions in reversed-phase on bonded silicas).	High
Packing Purity	Packing purity can affect separation as well as collection of purified analyte; trace metals in silica affects silanol acidity; residual monomers from polymeric packings or chemical reactants from banded silica-based materials can bleed, affecting collected analyte purity.	Moderate-to-High
Efficiency	Based upon particle size, mobile-phase linear velocity, and nature of phase chemistry (mass transfer); most important for complex samples, closely eluted compounds; often columns are run in an overloaded condition so potential column efficiency can be compromised.	Moderate
Mechanical Stability	Depending upon particle size, packing conditions, and operating conditions (mobile phase and flow rate), column packing can be subject to high mechanical stress; in large-diameter dynamic axial compression systems, mechanical stress can be significant; in systems where columns must be unpacked and repacked, good mechanical stability is required; particle strength is dictated by shape of particle (spherical, irregular); particle chemistry (silica, polymer-degree of cross-linking, other inorganic, monolith); pore volume; pore diameter; and treatment of particle; overall column stability affects operating cost.	Moderate
Chemical Stability	Dependent on particle chemistry (silica, polymer, other inorganic), phase chemistry (especially at pH values less than pH 2 and greater than pH 9 for silica-based type of bonding and bonding density), operating conditions (temperature, mobile phase including additives, manufacturing method (sol-gel vs. sil-gel for silica), regeneration, cleaning and sterilization conditions; phase bleed from packing causing purity and detection problems; overall column stability affects operating cost.	Moderate-to-High
Loadability	Affects throughput and analyte purity; overload is defined as amount of substance that can be injected onto a column that no longer permits the isolation of product at the desired level of purity or recovery levels; can have mass overload or volume overload; depending upon mode of operation, loadability is determined by surface area, pore size, pore size distribution (adsorption mode); bonded-phase coverage (reversed- and normal-bonded phase modes); ion exchange capacity (ion exchange; mode) and ligand density (chiral mode); relates to recovery.	High
Scability	Defines ability to scale-up separation from analytical to preparative to process scale; linear scale-up minimizes time required to optimize preparative separation; if same packing material is used for analytical and preparative systems, then scale-up should be easier; sometimes for cost reasons, larger particle sizes can be used in large-scale preparative and process environments; if different particle size material is from same batch or manufacturing process, separations should be similar.	Moderate
Recovery	Affects purity and yield; recovery is the mass of material eluted divided by the mass of material injected; usually expressed as percentage; high recovery most important for analyte of interest, but if contaminants remain behind, column performance and lifetime can be affected.	Moderate-to-High

to optimize their separations on a smaller monolith column and then scale up to industrial-scale production and purification. The company produces columns that can operate in an axial mode as normal chromatographic columns or in a radial mode for higher throughput. A recent paper showed that the performance of the CIM monolith in both modes is equivalent, and methods can be transferred from one to the other (6).

Swift polymeric monolith preparative

columns were introduced at Pittcon 2004 by Isco (Lincoln, Nebraska). To this point, Isco has focused on analytical-sized columns, and these hydrophilic polymethacrylate-based columns are the company's first entry into the preparative monolith market. The columns are scalable so that methods can be transferred easily. The methacrylate Swift Preparative columns come in four chemistries: strong anion, strong cation, weak anion, and weak cation. A fifth chemistry is based

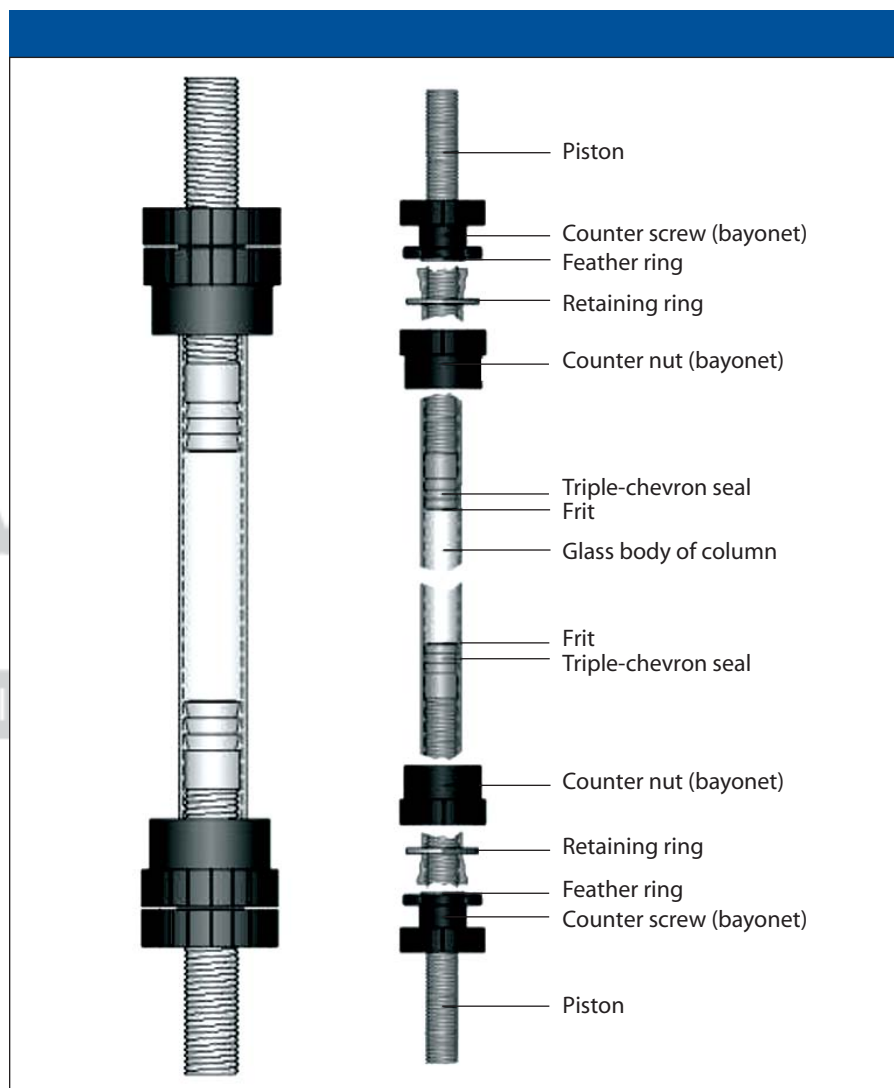
upon a PS-DVB monolith and is termed Swift RP-ST-1X (formerly the RP-pep). These biocompatible columns are offered in diameters of 16, 27, 34, and 50 mm and in lengths ranging from 30 to 250 mm. For these monoliths, Isco has specially designed a high-pressure glass column in an aluminum protective housing for safety protection. Both of the endpieces are adjustable so that users can tighten the pistons easily to minimize the dead volume and maintain the column efficiency

throughout the life of the column. In addition, the column shield has two observation windows to facilitate viewing of the monolithic column as it is being used. These polymeric columns are recommended for the rapid purification of biomolecules.

Merck KgaA (Darmstadt, Germany) is the lone participant in the silica-based monolith market and it, too, initially focused on conventional analytical and, more recently, capillary columns. It now has joined the preparative monolith world. The Chromolith PrepRod column has a 25-mm inner diameter and a length of 10 cm. An underivatized silica version and a C18 chemically bonded version are the company's initial offerings. In a study to access the suitability of silica-based monoliths for high-throughput semipreparative purifications, Goetzinger and colleagues (7) found that Chromolith columns have slightly less volume loading capacity compared to packed porous monomeric bonded-phase columns. However, for mass loading sample capacity, the two were similar. Due to their higher permeability, longer monolith columns can be used without significant increases in backpressure resulting in a higher overall loading per column.

### Preparative Column Hardware Requirements

Prepacked, standard preparative columns with compression fittings are available from most suppliers. Most of these prepacked columns have been filled using optimized packing procedures so that column life is often adequate to satisfy most user needs. Cartridge preparative columns with reusable endfittings also are available for easy column replacement. Because the endfittings can be used again and again, column replacement costs are less than fixed endfitting preparative columns. One such design that also has an integrated guard column is pictured in Figure 1. Flanged column hardware with dispersion plates or special geometry to spread the injected sample over the diameter of the packing, especially important for very large diameter preparative columns, is available from several suppliers. If a user has a special need, there are companies such as ModCol (Hesperia, California), now a division of Grace Vydac, that will custom slurry pack materials into almost any size compression fitting style-, flange style-, or spring-loaded-style preparative column. Columns with inner diameters as large as



**Figure 2:** Cross section of Econoline axial compression glass column. (Courtesy of Essential Life Solutions.)

100 mm can be custom-packed with the company's own packing material, packing material from other manufacturers, or with the user's own custom packing. Most companies in the preparative market will pack custom sizes of their own preparative materials.

On the other hand, there also are users who prefer to pack their own columns. Manufacturers have responded by offering the packing hardware itself and the bulk packing materials to fill the columns. The packing hardware that has proven to be the most popular is that which employs dynamic axial compression. Prepacked radial-compression columns still are available, but they require a special housing apparatus for their operation, they have proprietary plastic column hardware, and users cannot pack their own columns. Axial compressed columns are available in

glass as well as stainless steel and other popular hardware designs. An example of a glass column design is depicted in Figure 2. The Econoline column shown here is available from Essential Life Solutions (Boston, Massachusetts), which also offers axial compression columns of acrylic and glass construction in a wide variety of dimensions. The column pressure ratings are based upon the internal diameter. For example, a 5-mm i.d. column can withstand pressures as high as 90 bar, while a 50-mm i.d. column can withstand 25 bar. Users can fill the column with their favorite packing material, then screw down the movable threaded endfittings to tighten the bed. The threaded screws allow the height of the packed bed to be adjusted. Obviously, this design precludes the use of very small particles (5  $\mu\text{m}$  or less).

For high-pressure operations with small-

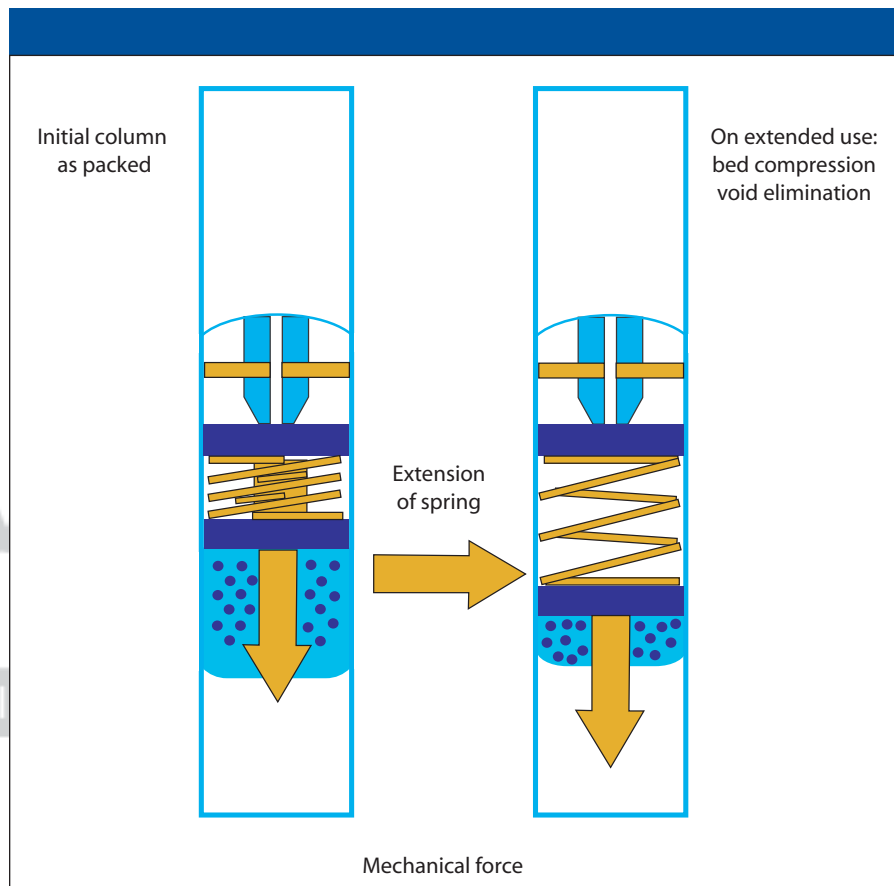


**Figure 3:** Example of high-pressure axial compression stainless steel column. (Courtesy of EMD Chemicals.)

particle preparative packings, many companies have introduced stainless steel versions of an axial compression system. These are particularly useful for certain packings with which, under high-pressure operation or under unfavorable chromatographic conditions, the bed might settle or some of the packing material might be eroded.

### ***In addition to prepacked columns, most manufacturers offer their materials in bulk quantities.***

Thus, the ability to recompress or further compress the packed bed can offer an easy alternative to replacing or repacking the preparative column. An example of high-pressure axial compression column is shown in Figure 3, which pictures a stainless steel flanged column with an axial compression screw device that can be used for both hard packing materials as well as soft gels; the latter of which still is popular in the biochromatography community. One of the more innovative designs in this area is the ModCol Axial Compression Spring technology, which automatically compensates for voids formed by bed settling, extending column life and improving column performance. As depicted in Figure 4, this technology utilizes a spring-driven compression mechanism instead of the traditional hydraulic system. The spring in the column keeps the pressure level constant and compensates for any deterioration of column packing material or the packed bed. As the volume occupied by the media gradually contracts, the spring in



**Figure 4:** Cross section of axial compression spring technology (see text for details). (Courtesy of ModCol, division of Grace Vydac.)

the column expands, taking up the empty volume and preventing void formation in the packed bed.

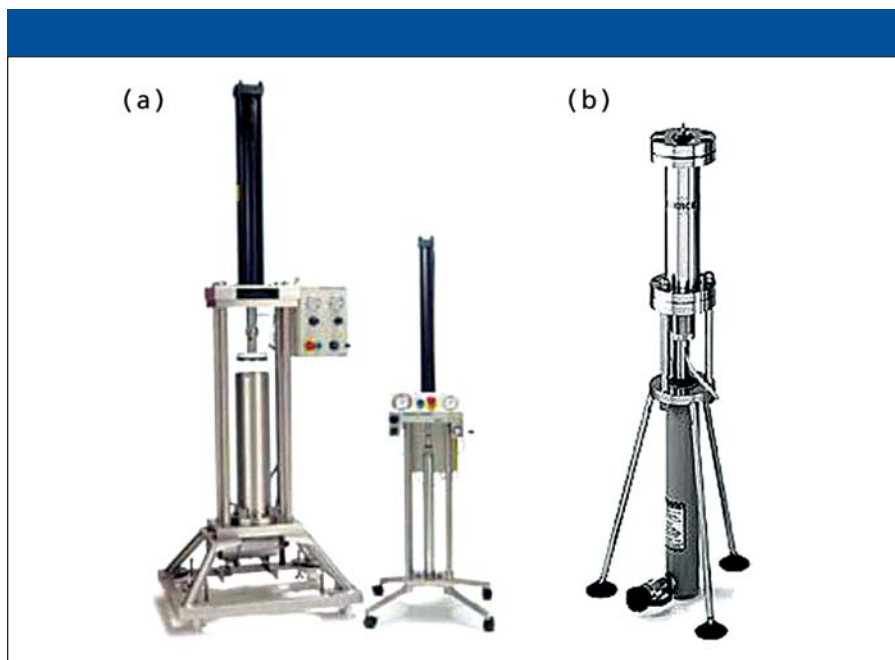
The larger axial compression type of columns then can be placed into a holder or preparative column stand for use in a laboratory or pilot plant. Two such configurations of commercially available products are shown in Figure 5.

#### **Bulk Preparative Packing Materials**

Because some of those involved in preparative chromatography have purchased a dynamic axial compression system, there is a market for bulk packing materials that matches the analytical or semipreparative column that might have been used for the initial method development studies. In addition to prepacked columns, most manufacturers offer their materials in bulk quantities. Thus, the scale-up process can be much easier to carry out. Several companies have introduced new lines of spherical preparative materials that are optimized for large-scale work. One such product offering is the PharmPrep CC (column

chromatography) by EMD Chemicals (formerly EM Science, Gibbstown, New Jersey), designed specifically for pharmaceutical purifications. This material is available in particle sizes of 40–63  $\mu\text{m}$  and the surface chemistry matches the company's LiChrospher 15- $\mu\text{m}$  material. Obviously, the resolution is reduced for the larger particle size material, but the selectivity between the two sizes is comparable.

In the bulk packings market, the bigger players often are chemical companies using their production scale to manufacture large quantities of bulk packing material. Aside from EMD Chemicals, companies such as Mitsubishi Chemicals America (White Plains, New York), Rohm and Haas (Philadelphia, Pennsylvania), Akzo Nobel/Eka Chemicals (Bohus, Sweden), Daiso (Osaka, Japan), Tosoh Bioscience (Montgomeryville, Pennsylvania), and Grace Vydac offer drums of material if the need arises. An example would be the Amberchrom resins from Rohm and Haas. The Amberchrom CG resins have been proven commercially for many years and are used in many pharmaceutical applica-



**Figure 5:** Schematics of large-scale self-packing preparative column hardware. (a) Varian's 100/150 mm and 41/77 mm RamPak systems (courtesy of Varian Associates, Walnut Creek, California) and (b) EMD Chemical's Self-Packer column-packing stand. (Courtesy of EMD Chemicals.)

tions. Most of these are based upon ion-exchange separations. Now that reversed-phase chromatography has extended its range of applications into the prep domain, the newest introduction the Amberchrom XT polishing grade PS-DVB resins (300-Å pore diameter) demonstrate improved selectivity for difficult reversed-phase chromatography separations when compared with silica packings. They offer high capacity and high flow rates in medium- to high-pressure production applications. As with many polymeric materials, they can be treated with harsh reagents such as sodium hydroxide, while silica has stability limitations at high pH. Available in two particle size ranges (20 and 30  $\mu\text{m}$ ) Amberchrom XT resins are compatible with most organic solvents, dilute acids, and bases. Amberchrom XT media have been used in purifying hydrophobic and polar peptides, small proteins, and oligonucleotides. They also are suited for therapeutic proteins such as insulin.

Bio-Rad Laboratories (Hercules, California) also has a new line of ion-exchange resins for bioseparations. Their macroporous UNOsphere packings (120- $\mu\text{m}$  average particle size) are based upon an acrylamide-vinyl copolymer and have been functionalized with either a quaternary amine (anion) or a sulfonic group (cation). They are recommended for the isolation of biomolecules from crude feedstock, one of

the more demanding chromatographic steps in the downstream process. The dynamic binding capacity of the UNOsphere Q is 125–180 mg BSA/mL in the linear velocity range of 150–1200 cm/h. For the UNOsphere S, it is 30–60 mg IgG/mL in the linear velocity range of 150–1200 cm/h. The high binding capacity at the high linear velocities is attributed to the macropores that are greater than 2000 Å, leading to fast-binding kinetics and high binding capacities.

### Flash Chromatography

Flash chromatography is a throwback to the old days of preparative chromatography, when chromatographers spent hours filling and unpacking glass columns for separation of crude synthetic mixtures or natural products. Then, gravity-feed systems were the norm with huge reservoirs placed overhead and column effluent collected manually (or if one was lucky, with a crude time-based fraction collector). Today, convenient prepacked flash columns and cartridges are available with predetermined masses of packing and with various dimensions. In fact, there are several companies that provide fully automated or nearly automated flash chromatography instruments to make the whole process easy and convenient. Examples of companies who have focused instrumentation products for flash chromatography include Isco's Combiflash systems (Lincoln, Nebraska), Arg-

onaut's FlashMaster (Foster City, California), and Gyan's JaiFlash (Genoa, Italy).

To fill the need for replacement flash columns, column companies have responded with prepacked flash columns to fit these and other flash chromatography instruments. A replacement flash column-cartridge market has sprung up, as shown by Varian's Mega Bond Elut Flash cartridges (Palo Alto, California) as replacements for Argonaut's FlashMaster. Varian's MetaFlash cartridges and Phenomenex's Flash Chromatography Cartridges (Torrance, California) are designed as replacements for Biotage's purification system (Charlottesville, Virginia).

Some companies have introduced generic flash chromatography columns such as the Chromabond Flash Cartridges from Macherey-Nagel (Dueren, Germany). These columns are packed with 10–50  $\mu\text{m}$  irregular or spherical packings with more than 30 different phase chemistries. These phases match the company's thin layer chromatography (TLC) plates so that method development and purity checks can be made during the flash chromatography experiments. A product brief that describes the use of TLC as an aid to develop methods for flash chromatography and choose effective solvent compositions is available from Argonaut (8).

### Preparative Supercritical Fluid Chromatography

Supercritical fluid chromatography (SFC) has been available for many years and has had its ups and downs in the analytical separations world. However, SFC has found a niche in preparative chromatography. Instrumentally, the technique can be performed with systems very similar to those used for conventional HPLC. Instead of pumping organic solvents, liquid carbon dioxide is the favored supercritical fluid and the pumping system requires only minor modification. The preparative SFC technique is fast, easy to perform, and collected fractions are recovered easily with high purity. The mobile phase, carbon dioxide, is converted to the gaseous state upon exiting the supercritical conditions leaving the analyte in a pure state. Occasionally, because carbon dioxide is a relatively nonpolar mobile phase, a polar dopant is needed to affect the mobile polarity. However, a small amount (less than 10%) is often sufficient. Thus, the amount of organic solvent needed for the purification is reduced dramatically compared with preparative LC. Although many

of the columns used for semipreparative and preparative LC are the same as those used in HPLC, several companies have specialized in offering their columns for SFC. Two that come to mind are Chiral Technologies (Exton, Pennsylvania) and Princeton Chromatography (Cranbury, New Jersey). Chiral Technologies focuses on SFC chiral columns, while Princeton Chromatography caters to the more general pharmaceutical market.

With preparative SFC, when dealing with polar compounds, stationary phases such as silica, cyano, diol, and amino are useful. Papers by Caldwell (9,10) showed that 2-, 3-, and 4-substituted pyridine amide and urea phases could be used to expand the selectivity of SFC. For acids and neutral compounds, method development for SFC is relatively straightforward, but for basic compounds, interaction between the amines and carbon dioxide, as well as the interactions with the surface of silica-based packings, results in severely spread or strongly sorbed peaks. Cox and Stringham (11) found that the addition of ethanesulfonic acid to the mobile phase alcohol modifier and sample diluent allows the separation of ethanesulfonic acid salts of primary, secondary, and tertiary amines as well as amino acids, amino acid esters, and beta-blockers. Of course, for the collected samples, the ethanesulfonic acid would have to be removed.

In the preparative separation of enantiomers and diastereomers, SFC has found widespread use. Biba and colleagues (12) reported on the use of a tandem column system to separate diastereomers and other compounds with multiple stereocenters. They modified a commercial SFC system that allowed software-based selection of 25 tandem-column arrangements and 10 single-column arrangements. The system simplified method development for multicomponent chiral separations.

## Conclusions

New developments in preparative chromatography continue to appear. For example, preparative monolith columns that offer advantages in their flow characteristics now have joined conventional particulate packings. Specialized instruments for semipreparative and preparative scale HPLC separations now make the job of fraction collection and method development easier. Flash chromatography products have supplanted the glass columns of yesteryear. SFC also has found a niche in the pharmaceutical purification market,

especially for chiral compounds. Preparative chromatography continues to look bright as the need for biopurification of proteins and for new pharmaceutical compounds is expanding.

## References

- (1) R.E. Majors, *LCGC* 22(5), 416–428 (2004).
- (2) R.E. Majors, *LCGC* 22(3), 230–242 (2004).
- (3) B.L. He, J.T. Lee, and T. E. Beesley, paper P-0305, HPLC 2004, June 14–20, 2004, Philadelphia, Pennsylvania.
- (4) C.V. McNeef, P.W. Carr, D.R. Hawker, D.R. Stoll, and A. Subramanian, paper 21700-2800P, Pittcon 2004, March 7–12, 2004, Chicago, Illinois.
- (5) F. Kanda, paper 1700-5300P, Pittcon 2004, March 7–12, 2004, Chicago, Illinois.
- (6) P.M. Zmak, H. Podgornik, J. Jancar, A. Podgornik, and A. Stancar, *J. Chromatogr., A* 1006, 195–205 (2003).
- (7) B. Zhang, H. O'Donnell, and W. Goetzinger, paper L-074, HPLC 2004, June 14–20, Philadelphia, Pennsylvania.
- (8) [http://www.argotech.com/FMDemo/pdf/flash\\_bookprintV8.pdf](http://www.argotech.com/FMDemo/pdf/flash_bookprintV8.pdf).
- (9) W. Caldwell, paper 15700-600P, Pittcon 2004, March 7–12, 2004, Chicago, Illinois.
- (10) W. Caldwell and J. Caldwell, paper P-2601, HPLC 2004, June 14–20, 2004, Philadelphia, Pennsylvania.
- (11) G. B. Cox and R. Stringham, paper L-1502, HPLC 2004, June 14–20, 2004, Philadelphia, Pennsylvania.
- (12) M. Biba, J. Gouker, C.J. Welsh, P. Augustine, G. Kath, K. Kline, and P. Hosek, paper L-0603, HPLC 2004, June 14–20, 2004, Philadelphia, Pennsylvania.

## Ronald E. Majors

*"Column Watch"*  
Editor Ronald E.

Majors is business development manager, Consumables and Accessories Business Unit, Agilent Technologies, Wilmington, Delaware, and is a member of

LCGC's editorial advisory board. Direct correspondence about this column to "Sample Prep Perspectives," LCGC, Woodbridge Corporate Plaza, 485 Route 1 South, Building F, First Floor, Iselin, NJ 08830, e-mail [lcgcedit@lcgmag.com](mailto:lcgcedit@lcgmag.com).



## Erratum

In the May "Column Watch" (*LCGC* 22[5], 416–428), the peaks in Figure 3 were labeled incorrectly. The first and second peaks should have been labeled theophylline and caffeine, respectively.