A Review of HPLC Column Packing Technology
by Ronald E. Majors

Congratulations on American Laboratory's 35th anniversary. The history of HPLC and its column technology parallels the history of American Laboratory. The meeting that comes to mind in which HPLC finally had its reckoning was the Fifth Symposium on the Advances in Chromatography, Las Vegas, NV, in 1969. Up until that time, column liquid chromatography was not part of any symposium, but was merely a technique practiced by a few organic chemists purifying their synthetic mixtures. Indeed, most of the technical papers at this 1969 Zlatkis Symposium (as it was then nicknamed, having been founded by the late Albert Zlatkis, Chemistry Professor at the University of Houston, TX) were gas chromatography presentations with a session or two devoted to this new technique. However, the discussion sessions were where the action took place, such as lively forums on columns and detector technology. Most of those notables present were from the GC ranks who discovered a fertile new ground to explore. Even then, GC had achieved a certain level of maturity in terms of the theory and basic studies of band broadening, stationary phase technology, and injection techniques, although further developments in capillary technology were to come in the 1970s. After that pivotal meeting, most of the fundamental chromatography investigators turned their attention from GC to HPLC.

The column has always played a major role in the progress of HPLC. The major developments in the late 1960s that turned HPLC from being a slow, relatively insensitive separation technique were 1) the flow-through low-volume UV detector and 2) pellicular packings (also known as superficially porous or porous layer bead packings). The intent of this paper is to focus on the development of HPLC column packings from the early days until the present day. Since this is such a wide area, the article will center mainly on developments in particle morphology and column design. Modern column technologies that make HPLC one of the major tools of practicing chemists will then be discussed.

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<td>Driver</td>
<td>Improvement in quality of analysis</td>
<td>Reproducible columns; improved recovery, especially biological compounds; lower stationary phase surface activity, especially for basic compounds</td>
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<td>Cost of analysis</td>
<td>Improved column lifetime and stability; increased use of guard columns; narrow-bore columns with lower solvent usage; fast LC</td>
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<td>Smaller, more complex samples, trace analysis</td>
<td>Capillary and nanocolumns—specialty columns for selectivity improvements; increased use of column switching (2-D chromatography)</td>
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<td>Widespread use of LC-MS and LC-MS-MS</td>
<td>Capillary and nanocolumns; short, fast columns; packings with a wider range of solvent compatibility</td>
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<td>Smaller-diameter and shorter columns use less solvent—less toxic solvents around and lower disposal costs</td>
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Background

It has often been stated (or maybe overstated) that the column is the heart of the chromatograph. Without the proper choice of column and appropriate operating conditions, method development and optimization of HPLC separations can be both a frustrating and unrewarding experience. Column technology has developed alongside the development of HPLC instrumentation, sometimes actually outpacing it. For example, the technology to make and pack nanobore and capillary columns has been available for a long time, but due to nonoptimized instru-
and laboratory chip technologies are only the beginning.

The future—Informatics, informatics, informatics
There is no question that market-driven products that leverage informatics innovations and automation will be the backbone of our future. We will see informatics leveraging knowledge, reengineering processes, and ensuring compliance. Yes, compliance: Our life science and health-care priorities demand consumer confidence in the integrity of our data, product quality, and therapeutic claims. Informatics will lead the way.

1. Bioinformatics—merging biology, chemistry, and information technology. We must rationalize life sciences research and drug discovery. Far more complex disease mechanisms cannot be attacked with brute-force empirical techniques. Since data are inexpensive, we must extract knowledge and apply it to innovative therapeutic solutions.

2. Process informatics. Real-time information technology will be used in conjunction with process analytics to reengineer many processes to produce higher-quality products far more efficiently. Competitive and consumer pressures require greater productivity for all health-care products and services.

3. Compliance informatics. We are a risk-averse society that insists on rigid regulatory oversight to ensure safety and efficacy. The FDA and its regulated constituents agree that we must automate compliance, or compliance will become an insurmountable economic roadblock.

The tools we have are far more powerful, but our challenges are far greater, than ever before. Given the industry’s historical record of rising to new challenges, we can be very optimistic. Good luck to all.

Reference

Mr. Zente served as President and CEO of Waters Associates, now Waters Corp. (Milford, MA). He then cofounded and served as CEO of Zymark Corp. (Hopkinton, MA), and is currently cofounder and Chairman, VelQuest Corp., 35 South St., Hopkinton, MA 01748, U.S.A.; tel.: 508-380-7990; fax: 603-258-7962; e-mail: frank@velquest.com.
ments and the lack of real driving forces, these columns have sat idle awaiting the right opportunity to emerge. The recent successes in proteomics in which thousands of peptides from protein tryptic digests need to be separated and detected could only be accomplished by the use of capillary and nano-LC columns, often in two-dimensional configurations. These columns excel in sample-limited, high-sensitivity applications.

Table 1 outlines similar driving forces that have led and are leading to new types of stationary phases and column design. Many of the motivating forces for column developments are shared with other disciplines in analytical chemistry. For example, quality initiatives have propelled column manufacturers to improve the reproducibility and reliability of their columns, and today there are fewer complaints about column-to-column reproducibility than there were a decade ago. The increasing importance of biological samples has spurred the development of packings with biocompatible surfaces so that precious samples actually elute from the column. The current widespread use of LC-MS has encouraged developments in shorter and narrower-bore columns that have flow rate requirements within the optimum range of MS ionization techniques. The current importance of high-throughput LC columns based on conventional packings and recently on monolith technology is a direct result of the need for increased productivity.

Early history of HPLC particle development

Although theoretical predictions of improvements in column efficiency in liquid chromatography were known decades ago, the practical application of these predictions did not materialize quickly. The first practical work, which seeded the commercialization of liquid chro-

<table>
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<tr>
<th>Year(s) of acceptance</th>
<th>Particle size</th>
<th>Most popular nominal size (µm)</th>
<th>Plates/15 cm (approx.)</th>
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<tbody>
<tr>
<td>1950s</td>
<td></td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>1967</td>
<td></td>
<td>50 (pellicular)</td>
<td>1000</td>
</tr>
<tr>
<td>1972</td>
<td></td>
<td>10</td>
<td>6000</td>
</tr>
<tr>
<td>1985</td>
<td></td>
<td>5</td>
<td>12,000</td>
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<tr>
<td>1992</td>
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<td>3-3.5</td>
<td>22,000</td>
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<tr>
<td>1998*</td>
<td></td>
<td>1.5*(pellicular)</td>
<td>30,000</td>
</tr>
<tr>
<td>1999</td>
<td></td>
<td>5.0 (Poroshell)</td>
<td>8000**</td>
</tr>
<tr>
<td>2000</td>
<td></td>
<td>2.5</td>
<td>25,000</td>
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<tr>
<td>2003</td>
<td></td>
<td>1.8</td>
<td>32,500</td>
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*Nonporous silica or resins.
**For protein MW 5700.
matography, focused on gel chromatography—the forerunner of modern size exclusion chromatography. The research work of Porath and Flodin\(^1\) in cross-linked dextrans, and of Moore\(^2\) in polystyrenes pointed the way to the commercial development of Sephadex (Pharmacia, Uppsala, Sweden, now part of Amersham Biosciences, Chalfont St. Giles, Bucks, U.K.) and Strygell (Waters Associates, Milford, MA), respectively. However, by today's standards, these packings were rather large particles, and the low degree of cross-linking and structural rigidity did not permit their use in high-pressure systems.

Table 2 provides a historical overview of the development of stationary phase particles used in HPLC. Prior to the advent of HPLC, low-pressure, gravity-fed columns with large, irregularly shaped porous particles such as silica gel or alumina were the norm (Figure 1a). These particles were sorted into suitable size ranges by sieving. Due to their high surface area, typically several hundred square meters per gram, these porous particles had excellent sample capacity. However, the large particle sizes gave poor column efficiency. When one tried to speed up the separation by pressuring the column and thereby obtaining a higher flow rate, the solute mass transfer into and out of these large porous particles gave even broader peaks, resulting in further loss of resolution. The loss in resolution was caused by a loss of efficiency. The effect of particle size and linear velocity, \(v\) (proportional to flow rate), on column efficiency is clearly demonstrated in Figure 2.\(^4\) The figure, often referred to as a van Deemter plot, shows efficiency (H or height equivalent to a theoretical plate [HETP]) versus mobile phase linear velocity for a series of porous, silica-packed columns. The largest porous particle studied was a 45-\(\mu\)m particle, even smaller than those used in the gravity-fed columns. From Figure 2, one can easily see why smaller and smaller particles became desirable: increased efficiency (lower H values) and flatter van Deemter curves.

Prior to the reestablishment of porous particles in the early 1970s, an intermediate particle configuration had some success. Based on theoretical predictions of Purnell\(^5\) and Golay\(^6\) and the experimental gas chromatography results in Horváth's Ph.D. thesis,\(^7,8\) ion exchange and alumina pellicular packings for HPLC were introduced by Horváth and Lipsky,\(^9,10\) and silica particles by Kirkland\(^11\) at the 1969 Zlatkis Meeting, referred to above. These particles had a thin porous film of stationary phase deposited over a spherical solid glass bead with a particle diameter of around 40 \(\mu\)m in diameter (Figure 1b). Solute molecules could diffuse into and out of this thin porous layer, usually 1–3 \(\mu\)m thick, thereby demonstrating improved efficiency. In addition, these superficially porous particles had somewhat flatter H-v curves than the larger porous packings. The Corasil II (Waters) curve shown in Figure 2 was typical of a superficially porous packing (37–50 \(\mu\)m particle size range) with a silica gel surface (surface area

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**Comparison of Early Packing Types in LC**

![Comparison of Early Packing Types in LC](image)

**Comparison of Current Packing Types in HPLC**

![Comparison of Current Packing Types in HPLC](image)

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**Figure 1** Pictorial comparisons of liquid chromatography column packings (not to scale). a) Large, totally porous particle (100+ \(\mu\)m) (normally irregularly shaped); b) superficially porous particle (50 \(\mu\)m) (also called pellicular or porous layer bead); c) microparticulate totally porous particle (5 \(\mu\)m); d) perfusion particle (12 \(\mu\)m); e) nonporous silica (NPS) or nonporous resin (NPR) (1-5 \(\mu\)m); f) Poroshell (Agilent Technologies) particle (5 \(\mu\)m).
14 m$^2$/g). Columns were usually dry-packed, since the dense glass beads permitted vibration analogous to techniques used in those days to pack GC columns. Columns were typically 50–100 cm in length and 2.1 mm i.d. However, due to the small amount of stationary phase (typical silica surface areas were a few square meters per gram of packing), the sample capacity was greatly reduced relative to older porous packings. This low capacity limited their usefulness for preparative applications and for trace analysis with low-sensitivity detectors (i.e., refractive index detector). Even so, in the late 1960s and early ’70s, these packings became the rage, and many researchers turned to HPLC to solve their problems where GC had difficulties, namely nonvolatile or thermally sensitive compounds. As will be seen later, it is of interest to note that, even in modern times, workers have returned to the superficially porous packing concept to improve stationary phase mass transfer.

The use of smaller, porous particles (Figure 1c) overcame the limitation of the porous layer beads, but the unavailability of commercial quantities of narrow particle size distribution, small porous packings, and lack of packing techniques for them precluded their use. Dry packing such particles with diameters less than 20 μm was extremely difficult due to static charge buildup, but the work of Huber$^{12}$ showed that analysts could occasionally obtain a high-efficiency column with careful hand packing and tamping using a small PTFE-tipped rod.

Majors$^{13}$ successfully and reproducibly applied balanced-density slurry techniques to an irregular small-particle silica gel (5–10 μm) supplied by Merck KGaA (Darmstadt, Germany); this packing method and the availability of larger quantities of reasonably priced silica gel packing enabled the commercial production of microparticulate packed columns. The initial commercial product, called MicroPak (Varian Associates, Walnut Creek, CA), packed in 2.1-mm-i.d. columns provided HETP values in the 0.1-mm range. Kirkland,$^{14}$ then with DuPont Instruments (Wilmington, DE), developed Zorbax (now available from Agilent Technologies, Wilmington, DE), a spherical silica-gel material that was a suitable base material for further developments. In May 1972, Majors published his first review article on HPLC packing materials in American Laboratory.$^{15}$ Only one commercially available microparticulate packed column was noted in this review. After that initial review, tens of column companies entered the market to offer their own versions of microparticulate columns. Initially, 7- and 10-μm particles were the most popular. Through the following years, 10 μm gave way to 5 μm as the most widely used particle size. Irregular particles gave way to spherical particles, which are the only type of packing available for analytical applications today.

Throughout the 1980s, not much development work was devoted to the base silica materials but more to improving bonded phase chemistry, especially specialty bonded phases for chiral compounds; biological compounds, such as proteins and nucleotides; environmental compounds, such as polynuclear aromatic hydrocarbons; and ion chromatography packings. Chromatographers were quite happy using 5-μm silica-based bonded phases, and mostly complained about column-to-column and batch-to-batch reproducibility.$^{16}$ Some users turned to the use of 3-μm particles, but found that columns with these particles did not always perform as well as expected and appeared to plug more than their 5-μm counterparts.

**Particle and column developments in the last decade**

**Perfusion packings**

Even though rigid column packings had become very useful in the 1980s, there were still certain areas in which improvements were needed, particularly in the application of HPLC to large biomolecules. The original soft biocompatible gels, such as Sephadex and BioGel (Bio-Rad Laboratories, Hercules, CA), still commanded a great deal of attention, even though when subjected to high pressure they tended to collapse and thus were limited to low-pressure applications. Silica-
based packing materials with 300-Å pores became available, and these products began to see some success in the separation of proteins. The large molecules could diffuse into and out of the large pore openings. Since diffusion coefficients were very large, column efficiency tended to be lower than for small molecules. At high flow rates, proteins tended to broaden even further and thus high-speed, high-efficiency protein separations were difficult to achieve.

A major development in the separation of biomolecules was the introduction of perfusion chromatography by Afeyan and coworkers, the resulting packing material, Poros, was commercialized by Perceptive Biosystems (Cambridge, MA, now part of Applied Biosystems, Foster City, CA) in the late 1980s. A simplified pictorial representation of a perfusion packing is shown in Figure 1d. Compared to porous packings, the perfusion packings were claimed to consist of two different types of pores: diffusive pores and through-pores. The diffusive pores were the same type present in porous packings and provided the sorption capacity; the through-pores allowed a portion of the mobile phase to pass through the packing itself, thereby increasing the rate of mass transfer in the stationary phase. In the typical porous packings, almost all of the flow went around the particle. Thus, for perfusion packings, the solute spent less time in the stationary phase undergoing the mass transfer process and eluted as a narrower peak. In actuality, the entire process is a combination of diffusion and convection.

The cross-linked polystyrene-divinylbenzene Poros particles were somewhat larger than typical packings of the day, with average particle diameters over 10 μm. For large biomolecules, these packings gave adequate efficiency and displayed good sample capacity. Even today, they are useful in preparative and process chromatography. Since they are more rigid than the earlier polydextran gels, they can be used at higher flow rates and still display flat H–V curves. A strong attribute of the perfusion packings is the observation that through-pores, when coupled with diffusive pores, gave rise to improved performance; their larger particle size gave rise to low-pressure drops. These features were extended in the development of monolith technology (see monolith section).

Pellicular and superficially porous packings revisited

In the late 1980s to early 1990s, pellicular packings were revisited, but these newer versions greatly reduced particle sizes compared to their predecessors. The commercial introduction of nonporous silica (NPS) and nonporous resin (NPR) with particle diameters in the 1.5–2.5 μm range (Figure 1e) gave users another approach to high-speed biomolecule separations. The thin porous layer of bonded phase or ion exchange functionality deposited onto a small glass bead allowed much faster separations than the perfusion materials, but the small stationary phase capacity generally precluded their use in preparative separations. Although many fast separations were demonstrated for small molecules in short columns (~50 mm and less), in practice, there were few advantages over small porous particles of 3 μm in diameter. The reason for this smaller difference in performance was that small molecules have higher diffusion coefficients than large biomolecules and thus have higher rates of mass transfer. Also, due to their small particle sizes, columns packed with NPS and NPR resulted in much higher pressure drops than observed with the 3-μm packed column (remember pressure is inversely proportional to d³); thus, short columns had to be used.

![Figure 3](image-url) Comparative gradient separations of a protein tryptic digest on a Poroshell and a totally porous silica column. Upper chromatogram: Poroshell 300SB-C18, 2.1 x 75 mm, 5 μm. Lower chromatogram: Zorbax 300SB C18, 2.1 x 150 mm, 5 μm. Conditions—mobile phase: solvent A = 95% water/5% acetonitrile with 0.1% trifluoroacetic acid (TFA); solvent B = 95% acetonitrile/5% water with 0.07% TFA. Flow rate (upper chromatogram): 1 mL/min; gradient (upper chromatogram): 0–40% B in 12 min. Flow rate (lower chromatogram): 0.208 mL/min; gradient (lower chromatogram): 0–100% in 120 min; temperature: 70 °C; detection: UV, 215 nm; sample: bovine serum albumin, 70 μmol digested with trypsin for 15 hr.
The Poroshell column (Figure 1f) described by Kirkland and commercialized by Agilent Technologies has characteristics similar to the NPS, but due to its larger particle size (dp = 5 μm), the pressure drop is much lower. The porous layer is formed on a solid silica core material and hence is quite stable. Because of the superficially porous layer of stationary phase, H-v curves are very flat, and high-speed separations of biomolecules can be achieved at a relatively low pressure drop. The thin layer of stationary phase silica is derivatized with alkyl-bonded moieties such as C3, C8, or C18, providing rapid, reversed-phase separations of proteins and peptides. Figure 3 compares the rapid separation of a 15-hr tryptic digest of 70 pmol of bovine serum albumin on a Poroshell StableBond C18 (SB C18) column (2.1 × 75 mm) (Agilent Technologies) to the same separation performed on a longer totally porous Zorbax StableBond C18 column (2.1 × 150 mm) (Agilent Technologies). The separation conditions were adjusted to achieve approximately the same number of peptide peaks. Note that the separation time on the superficially porous column was a tenth of the separation time on the totally porous column.

**Monoliths**

One of the more exciting HPLC column innovations in the last several years has been the commercial development of monoliths. Monoliths are columns that have the stationary phase cast (synthesized) as a continuous homogeneous phase (much like concrete in a mold) rather than packed as individual particles. Both silica gel and polymeric monoliths are available. The monolith phases have some similarities to the perfusion phases. For example, silica monolith consists of through-pores (called macropores) approx. 1–2 μm in width and diffusive pores (called mesopores) approx. 120 nm. These phases can be derivatized with the same bonded phases available in microparticulate columns. The silica columns are available as rods that are clad in polyetherether ketone (PEEK) tubing. The efficiency of these columns, commercialized by Merck as Chromolith™, claimed to be equivalent to a 3–5 μm silica particle, but an important feature is that the pressure drop is considerably lower than a microparticulate column with the same particle size. Therefore, higher flow rates may be used and longer columns may be connected in series without overpressuring the HPLC pump. The monoliths display a significant improvement in separation impedance (plates per unit time and pressure) over microparticulate columns.

Figure 4 is a series of gradient separations of beta-blocker drugs on a 4.6 × 50 mm Chromolith SpeedROD RP-18e monolith column with flow rates increasing to 9 mL/min with a column pressure drop of only 1060 psi, well below the output pressure of most modern HPLC pumps. Currently, only reversed-phase silica monoliths are available, but that is expected to change as new phases are requested. The silica monoliths...
have mainly been used for the separation of small molecules, but recently biomolecular separations have been presented.\textsuperscript{22}

Polymeric monoliths have also been commercialized. Polymeric monoliths are available as cross-linked polymethacrylates and polystyrene-divinylbenzene that are functionalized with ion exchange, affinity, hydrophobic interaction, and reversed-phase groups. They are offered in diameters as small as 200 μm (capillary dimensions) and with volumes as large as 8 L (process dimensions). The polymeric monoliths have had their biggest success in the separation of biomolecules. The monoliths have been fabricated in disks, sheets, rods, and in disposable cartridges. Those interested in monolith technology may refer to a monograph\textsuperscript{23} and recent review article.\textsuperscript{24}

**Other modern packing materials**

Other nonsilica-based packing materials have been introduced in the last several years. The scope of this article is not to discuss all of these in depth, but to provide an overview of new phases that may have some advantages for use in the laboratory.

Although reversed-phase polymeric materials have been around a long time, they have never gained popularity. Polymeric packings never provided the efficiency of silica-gel packings. For reversed-phase chromatography, an equivalent-size polymeric particle packed into an HPLC column will provide about a third of the plate count for small solutes compared to silica particles. For ion exchange and size exclusion chromatographic separations, polymeric materials offer stability and pore size advantages, respectively.

Among the polymeric packings, polystyrene-divinylbenzene and polymethacrylate are among the more popular. Most often users turn to them when they are required to work at high pH (pH >9); silica gel has a finite solubility in water at neutral pH, and this solubility quickly accelerates as the pH is raised to 10 or 11. However, recent advances in bonding and synthesis chemistry have permitted silica-based packings to be used above pH 9, even as high as pH 12. One such synthesis is the inorganic-organic hybrid, where an alkyl silica monomer is polymerized to form
a phase containing inorganic siloxane bonds and organic silica alkyl bonds. The commercial version of this approach is the EXterra columns (Waters), which have proven to be more stable at high pH than typical silica-based packings. More recently, Wyndham and co-workers extended this chemistry to ethyl-bridged alkoxy silanes, which form the base material for the synthesis of new column packings. To extend the operating range of silica-bonded phases, Kirkland and co-workers (Agilent Technologies) used a special bidentate-bonded reversed-phase ligand that is anchored at two adjacent silanols. When this phase is combined with a high degree of end-capping, the resultant packing can withstand high pH for extended periods of time.

Zirconia (ZrO2) has some properties that make it attractive as an HPLC packing, particularly its stability. It can be produced in monodisperse, porous spherical particles, and for many separations can provide the efficiency of silica gel. Its high pH stability is excellent, and there are no surface silanols for amine interactions. However, it does possess hard Lewis acid sites and has a strong affinity for Lewis bases (e.g., hydroxyl, phosphate, and carboxylate). Therefore, competing anions must be added to the mobile phase for successful separations of these compounds. Zirconia can be coated with a variety of phases, including polybutadiene and pyrolytic carbon; it has proven useful for the separation of diastereomeric compounds and stereoisomers. It can also withstand high temperatures and has been used up to 200°C. For more information on these columns, consult Ref. 29.

Small particles are experiencing comeback in high-throughput HPLC. Small particles packed into short columns provide rapid separations with only minimal losses in resolution compared to larger particles in longer columns. Thus, at a constant flow rate, if one goes from a 4.6 × 150 mm column packed with 5-μm particles to a 4.6 × 75 mm column packed with 3.5-μm particles, the same separation can be accomplished in half the time and with half the solvent. Figure 5 provides a visual comparison of the isocratic reversed-phase separation of four steroids using three different particle sizes packed into three different column lengths, each with the same internal diameter. Note that by using a 1.8-μm particle size in a 4.6 × 30 mm column run at a 2-μL/min flow rate, the separation time is a factor of 15 less than the more typical 4.6 × 250 mm column packed with 5-μm run at 1.0 μL/min. For such a short column, the pressure drop is around 200 bar for the 1.8-μm particles, well within the specifications of most HPLC pumps.

Although short columns with small particles provide rapid separations, the column plate number (efficiency) is not increased. To increase plate counts for more complex separations, long columns (greater than 50 cm) may be combined with small particles (~1 μm) to achieve greater efficiency. Jorgensen and co-workers (University of North Carolina, Raleigh, NC) have investigated ultrahigh-pressure chromatography, in which pressures in the tens of thousands of psi (thousands of bar) are required to pump mobile phase through relatively long columns packed with 1-μm particles, realizing plate counts in excess of 250,000 in less than 1 hr. Unfortunately, no commercial HPLC systems are available that can withstand the 50,000-psi pressures that these columns require.

Future directions in HPLC column and particle technology

Despite many new materials introduced in the last decade, silica gel-based packings have maintained their dominance and will continue to do so into the next decade. Silica monoliths have the potential to gain prominence, but costs will have to decrease and smaller diameters must be made available. Recently, 100-μm silica and polymeric monoliths made their appearance, although the main application of these columns will probably be in the handling of trace biological samples with mass spectrometric detection.

Polymeric monoliths seem to have a bright future in the process area for the purification of biomolecules. Monoliths have the advantage that column end frits are not required; thus, they may find use in capillary electrochromatography. New materials such as organic-inorganic hybrids and various composites could bring added advantages to expand the pH and temperature ranges, allowing novel parameters for method development and optimization. The current studies on "lab-on-a-chip" will result in packing materials that will be fabricated on the inner walls of these nanochannels. Conventional packings will have a difficult time when such small dimensions are encountered. The formation of monoliths inside of nanochannels is an easier way to synthesize these chip columns since monomers, cross-linkers, and porogenic solvent can be pumped into these tiny devices and polymerization initiated by thermal or UV irradiation. To be manufactured on a commercial basis, chip-to-chip reproducibility must be demonstrated.

The trend in column dimensions will continue toward the more gen-
eral use of smaller particles (3 μm) packed into short columns (75 mm and lower) when chromatographers realize that they do not need to use long columns with 5-μm particles. Conventional columns with internal diameters of less than 100 μm (called nanocolumns) are of interest to those studying proteomics. The small samples and low concentration of analytes strongly favor these miniature columns packed with small particle size packings. Mass spectrometry will be the main detection technique due to the sensitivity requirements for picomoles and less individual solute peaks injected onto the column. For samples in which mass is not limited, these columns will find less use since conventional HPLC instruments can be used, whereas the nanocolumns require matching instruments with extremely low dispersion characteristics.

References


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