

High-Performance SEC Column Technology

Although size exclusion chromatography (SEC) is a fairly mature separation technique, improvements are continually made in packing technology. Howard Barth and Greg Saunders review some of the basics of SEC and look at the current status of column technology, including developments for faster and higher resolution size separations.



The purpose of this paper is to review the desired characteristics of size exclusion chromatography (SEC) columns and to present highlights of recent advances in commercially available column technology. Unlike all other forms of chromatography, the separation mechanism of SEC, also referred to as gel permeation chromatography (GPC), is based strictly on the molecular size and shape of a solute with respect to the average pore size and geometry of the packing. The separation mechanism is shown schematically in Figure 1, and Figure 2 shows some scanning electron micrographs of typical SEC column packings. Figure 3 shows a typical SEC calibration curve in which the log of the molecular weight (MW) of a series of polymers is plotted against elution volume. Polymers that are too large to penetrate the pores of the packed bed are excluded from the packing pore volume (V_i) and are eluted in the interstitial volume of the column (V_0). As the MW or hydrodynamic volume of the polymer approaches the average pore size of the packing, the polymer penetrates more deeply into the pores, occupies more pore volume, and are eluted later. Smaller molecules that can freely diffuse into and out of the packing sample both the column pore volume and interstitial volume and, thus, are eluted at the total permeation volume ($V_T = V_i + V_0$) of a given column set. From the SEC elution profile of a polymer sample, its MW distribution can be determined from a calibration curve or using a MW-sensitive detector.

Desired SEC Column Characteristics

Because of the difference in separation mechanism between noninteractive SEC and high performance liquid chromatography (HPLC), where analytes are separated by their interaction with the packing material, there are certain limitations imposed on SEC columns that are not of major concern in HPLC, as summarized in Table I. The desired characteristics in SEC column technology are

high chromatographic resolution (efficiency and selectivity), broad molecular weight separation range, an easily fitted and interpolated calibration curve (log MW vs. elution volume), and packing inertness and ruggedness.

It is important to realize that a polymer sample is eluted only within the pore volume of an SEC column, which defines its molecular weight distribution (MWD). Although the trend in interactive HPLC is to use smaller-diameter columns, SEC columns of large pore volume are needed to maintain high pore volumes and, thus, MW accuracy and precision. As discussed in the following section, however, there are situations in which small-pore-volume columns are required, as in SEC-MS or for high-throughput analysis.

For optimum SEC resolution, the slope (D2) of the calibration curve (log MW vs. V_i) must be minimized. In other words, for increased resolution, the elution volume required to separate two polymers that differ by a decade difference in MW must be maximized (1). For increased column resolution, high V_i/V_0 values are required. However, producing highly porous packings of controlled pore size is difficult; furthermore, mechanical stability is compromised. In actual practice, using longer or more columns in series is a more practical approach for decreasing the calibration curve slope.

For optimum SEC resolution, both peak broadening and slope must be minimized. SEC columns that have high plate numbers might not provide sufficient resolution unless the slope of the calibration curve is sufficiently low. For typical high-performance columns and SEC systems, columns should be capable of generating $> 10^4$ theoretical plates to ensure a MW peak broadening error $< 2\%$ (2). With high-performance packings, this level can be realized by using $\leq 5\text{-}\mu\text{m}$ packings in $\leq 25\text{-cm}$ columns (3). Although with modern MW-sensitive detectors the influence of peak broadening is relaxed, high resolution is still an important consideration to achieve

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Table 1: Comparison of Separation Characteristics of SEC and HPLC

| | Separation mechanism | Parameters that influence column efficiency | Parameters that influence column selectivity |
|------------------|--|---|---|
| Interactive HPLC | chemical structural differences (enthalpic interactions) | particle size, column length, linear velocity, injection volume, column temperature | nature of stationary and mobile phases, and column temperature |
| SEC | molecular hydrodynamic volume and conformation differences (entropic interactions) | particle size, column length, linear velocity, injection volume, column temperature | packing pore size uniformity, and geometry, column V_i/V_0 , and log MW vs. V_r calibration curve slope (D_2) |

accurate MW data. For high SEC column efficiency, small particles are needed, as in the case of interactive HPLC. However, the lower particle size limit is dictated by the onset of polymer shear degradation for the analysis of ultrahigh MW samples (4). Typical packing sizes for SEC are in the 3–20 μm range, depending upon the upper MW being determined.

To maximize the resolution of an SEC separation, it is best to select a column or column set that encompasses only the MW range of a given sample. However, because many labs deal with polymers that cover either an unknown or a wide MW range, it is more convenient to employ “mixed-bed” columns, also referred to as “linear” columns, which typically can encompass three to five MW decades of separation for random-coil polymers. The upper effective MW range for typical SEC columns is about 10^6 g/mol, depending upon polymer conformation. With ultrahigh MW polymers, columns packed with larger particles and high-porosity frits are employed to avoid polymer shear degradation. At the low MW end, SEC columns are available that can separate fairly low MW solutes, although

interactive HPLC is better suited for the analysis of small molecules.

To prevent enthalpic interactions between sample and packing, potential adsorption sites must be absent from SEC packings. For the analysis of organosoluble polymers, cross-linked polymeric packings made from polystyrene (PS) and poly(divinylbenzene) (DVB) are commonly used with a wide range of organic solvents, such as tetrahydrofuran, toluene, chloroform, dimethylformamide, dimethylacetamide, dimethyl sulfoxide, N-methylpyrrolidinone, hexafluoroisopropanol, and trichlorobenzene. Hydrophilic polymeric packings, such as poly(vinyl alcohol), poly(acrylamide), or sulfonated polystyrene, find use with water-soluble polymers. Silica-based packings, either bare or silanized, can be used for the analysis of organo-soluble polymers, while hydrophilically modified silicas are used extensively for biopolymers and synthetic water-soluble polymers. For SEC of water-soluble polymers, however, there is no universal SEC system as water is capable of solubilising polymers containing both ionic groups and a considerable hydrophobic content. Column–mobile phase conditions must be tuned depending upon the chemical structure and ionic nature of the polymer. Selection typically is done empirically by adjusting and fine-tuning pH, ionic strength, ratio of aqueous/organic solvent, and temperature to

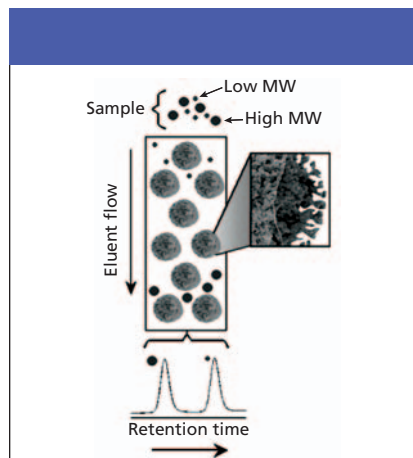


Figure 1: Schematic of the SEC mechanism.

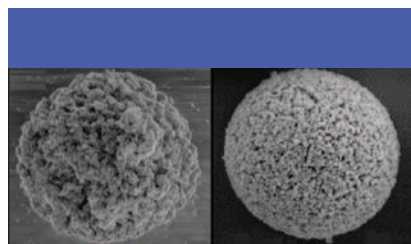


Figure 2: Scanning electron micrographs of typical SEC column packings.

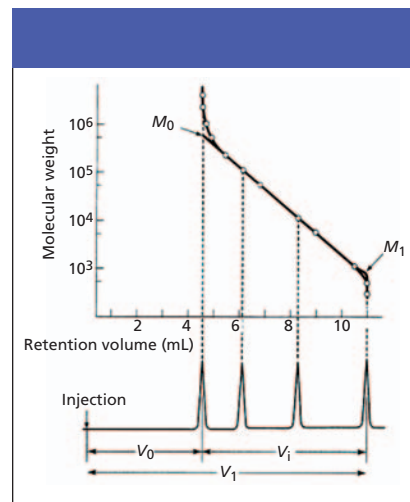


Figure 3: Typical SEC calibration curve in which V_0 is the interstitial volume, V_i is the pore volume, and V_t is the total column volume. M_0 and M_1 are the extrapolated exclusion and total permeation MW values of the column, respectively. (Adapted from reference 3.)

prevent packing interactions and unwanted intramolecular electrostatic interactions (5).

In general, silica-based packings can be used with a wide range of organic mobile phases and temperatures without altering particle morphology. However, silica-based particles are less stable in aqueous mobile phases, especially at extreme pH values and high electrolyte concentrations. Furthermore, silica packings are more acidic because of residual silanol groups, which is of concern when analyzing basic polymers. Silica-based packings generally have lower V_i/V_0 ratios (lower porosity) than polymeric packings, which leads to lower resolution than polymeric-based packings of comparable column efficiency. Finally, silica packings tend to have a considerably narrower molecular weight operating range than a polymeric analogue, reducing suitability to the analysis of many broad synthetic polymers.

New Packings for Organosoluble Polymers

SEC in organic solvents is by far the most common form of the technique, mainly due to the insolubility of the majority of polymers in aqueous solvents. An example of a typical organic SEC application is shown in Figure 4, the separation of a series of epoxy resins of increasing molecular weight. In this example, each sample contains a number of well-resolved oligomers as well as an appreciable polymeric content. Individual oligomers that are present in each of the samples can be readily identified.

The smallest PS-DVB packing particle size commercially available is 3 μm . These packings are limited to the SEC analysis of lower

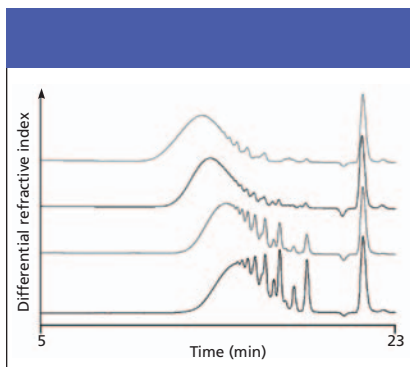


Figure 4: Separation of four epoxy resins on two ResiPore columns (Polymer Laboratories) with a 20- μ L injection volume and differential refractive index detection.

MW polymers because of the potential of shear degradation with high MW polymers. A number of manufacturers have introduced SEC columns of dimensions that are smaller in length than conventional columns that typically range from 7 to 8 mm i.d. and 250 to 300 mm in length. The advantage of a reduced column length is a faster analysis time and higher sample throughput compared to typical analytical columns. Also, columns of reduced internal diameter have been developed that have the benefit of employing less eluent and therefore increased compatibility with SEC-MS and applicability to limited sample amount. In addition, reduced consumption of expensive and environmentally damaging organic solvents is also a major advantage. However, with reductions in column length or internal diameter, resolution and MW accuracy can be compromised with reduced column efficiency and pore volume.

Individual pore size columns contain a single material with a relatively narrow distribution of pore size and therefore restricted molecular weight operating range. More common are the mixed-bed or linear columns, where several packings are combined in the column to extend the operating range and ensure a linear calibration is produced. Columns of this type are the most popular for size exclusion of broad polymers.

Showa Denko (Kawasaki, Japan) Series K-800 (300 mm \times 8 mm) are available in as many as eight pore sizes and linear columns and are available packed in tetrahydrofuran, chloroform, dimethylformamide, toluene, or hexafluoroisopropanol. Shodex GPC KF-400, K-400, and HFIP-400 columns are now available in 300 mm \times 4.6 mm dimensions packed with an 8- μ m PS-DVB. Column dimensions of Shodex GPC LF series, packed with PS-DVB 8- μ m particles, are 250 mm \times 4.6 mm for high resolution separations and 150 mm \times 6 mm for rapid analysis. For SEC of more crystalline polymers, such as

polyamides and polyesters, the mobile phase of choice is hexafluoroisopropanol. To meet this need, Showa Denko has introduced PS-DVB packings (Shodex HFIP-600 series) that are packed in hexafluoroisopropanol, also available with 3- μ m particles packed in 150 mm \times 6 mm columns.

The Shodex GPC KF-600 series consists of 3- μ m packings in nine pore sizes from 20 to >1000 Å in 150 mm \times 6 mm columns. This shorter, narrower column requires about one-third of the solvent usage and half the analysis time compared to the more conventional sizes. In common with most other commercially available PS-DVB columns, many types of eluents can be used interchangeably without swelling or shrinkage. Shodex GPC Linear LF-804 series of PS-DVB (6 μ m, 300 mm \times 8 mm) is designed to have a wide MW linear range and improved lower molecular weight linearity.

PS-DVB packings are also available from Tosoh Bioscience (Montgomeryville, Pennsylvania) that supplies eight pore sizes and two mixed beds. Depending upon pore size, packings are 5, 6, 9, 10, and 13 μ m packed in 300 or 600 mm \times 7.5 or 7.8 mm columns, as well as preparative 600 mm \times 21.5 mm columns. A high-temperature version, TSK-GEL HHT rated for 140 °C, is also available. Another approach to increasing the operating range of SEC packings is employed in the TSKgel Multipore HxL-M packing. This packing is designed with multiple pore sizes within a single particle, giving a wide operating range of several decades of molecular weight without the need to blend individual components. The company's Multipore approach apparently gives better linearity over a wide MW range.

Tosoh Bioscience's new generation TSKgel Super HZ are high performance SEC packings based upon small particle technology. The HZ products for nonaqueous SEC are packed with 3- μ m PS-DVB particles in 150 mm \times 4.6 and 6.0 mm columns with five pore sizes. The smaller pore sizes also can separate monomers, oligomers, and polymer additives. TSK-gel HHR line consists of 12 new columns (300 \times 7.8 mm) with a wide variety of pore sizes including four mixed-bed columns. The significant feature of the HHR columns is their tolerance to different solvents with minimal swelling. Some of the common mobile phases that can be used are tetrahydrofuran, chloroform, methylene chloride, dimethylformamide, dimethylsulfoxide, hexafluoroisopropanol, acetone, ethanol, and *o*-dichlorobenzene.

Polymer Laboratories (Church Stretton, Shropshire, UK) has five types of mixed bed

columns packed with 3-, 5-, 10-, or 20- μ m PS-DVB particles covering from two to five decades of MW separation. The company's 20- μ m packing has an exclusion limit of 40×10^6 and is specifically designed for the analysis of ultrahigh MW polymers. PS-DVB columns of 250 mm \times 4.6 mm dimensions are available in different particle sizes for reduced solvent consumption, and 300 mm \times 25 mm columns are available for preparative work. All of these columns are stable at temperatures as high as 220 °C. Polymer Laboratories also has PL HFIPgel columns available in hexafluoroisopropanol available in 250 mm \times 4.6 mm and 300 mm \times 7.5 mm columns for polyester and polyamide analysis. The new PlusPore column range makes use of a new technology to produce high pore size packings with improved resolution compared to conventional GPC materials. Four columns are available, OligoPore, MesoPore, ResiPore, and PolyPore, covering the range of molecular weight as high as 2×10^6 g/mol and in 250 mm \times 4.6 mm and 300 mm \times 7.5 mm column dimensions. The improved pore volume of these packings gives high resolution by reducing the slope of the calibration. These materials have the added advantage that only one type of material is packed in the column, removing the possibility of "dislocation" effects that can be observed at the overlap of calibration curves when combining individual pore size columns.

Polymer Standards Service (PSS) (Mainz, Germany) offers PS-DVB packings (PSS SDV) from 5 to 20 μ m in a range of pore sizes, column dimensions, and as mixed-bed columns. PSS offers a number of chemically different polymeric packings designed for specific sample types and mobile phase compatibility to prevent adsorption. For example, PSS GRAL packings can be used with both polar organic and aqueous mobile phases. PSS GRAM columns consist of polyester-based gels that are compatible with aqueous and medium polarity eluents. To achieve high-temperature stability, PSS has produced plasma-treated PS-DVB gels for enhanced stability at temperatures as high as 220 °C.

Waters (Milford, Massachusetts) maintains a complete line of PS/DVB 5- μ m packings (Styragel HR) in seven pore sizes as well as two mixed-bed columns packed in 300 mm \times 7.8 and 4.6 mm columns. PS-DVB 10- μ m packings (Styragel HT) rated for 150 °C are also available. Styragel HMW columns, consisting of 20- μ m packings and high porosity 10- μ m frits, are designed for the analysis of ultrahigh MW polymers.

Jordi Associates' (Bellingham, Massachusetts) SEC packings are prepared from DVB

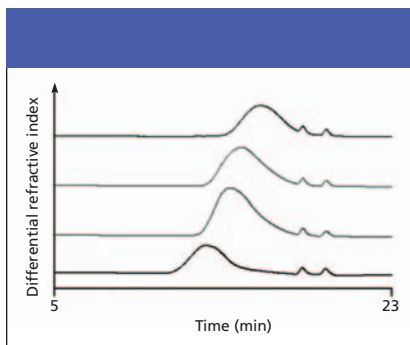


Figure 5: Separation of four samples of cellulose on two PL aquagel-OH MIXED 8- μ m columns (Polymer Laboratories) in a buffer of 0.2 M sodium nitrate, 0.01 M monobasic sodium phosphate adjusted to pH 9 with a 200- μ L injection volume and differential refractive index detection.

rather than PS-DVB. DVB packings are claimed to have greater stability and larger pore-volumes. These 5- μ m packings are available in five pore sizes, as well as a mixed bed, and in different column configurations. BioChrom Labs (Terre Haute, Indiana) has introduced Hydrocell GPC 3000 HS, a PS-DVB packing of 7–13 μ m available in columns of 300 and 150 mm \times 7.8 mm.

New Packings for Biopolymers and Water-Soluble Polymers

SEC separations in aqueous media are becoming more common for two reasons. Firstly, researchers are developing more materials that are water-soluble in the drive to reduce the ecological impact of polymers and to develop materials for biodegradable applications. Secondly, analysts are increasingly adopting aqueous methodologies where possible to reduce the need to handle unpleasant solvents and chemicals. This has led to a steady rise in the use of aqueous SEC. In a typical example of an aqueous SEC separation, Figure 5 shows the separation in aqueous media of a series of cellulose materials of increasing molecular weight. Careful modification of the eluent is required in this particular example to ensure that a purely SEC separation is obtained.

Tosoh Bioscience produces silica-based (TSK-Gel SW) and polymeric-based (TSKgel PW) packings. TSK-Gel SW is available in three pore sizes (125, 250, and 450 Å) and 5-, 8-, 10-, 13-, and 17- μ m particle sizes. Column dimensions are 15, 30, and 60 cm \times 7.5 and 7.8 mm, as well as 60 and 30 cm \times 21.5 and 55 mm for preparative separations. TSK-Gel PW packings are prepared via copolymerization of ethylene glycol and methacrylate. Pore sizes are <100, 125, <200, 200, 500, 1000, >1000 Å and particle sizes range from 6 to 25 μ m. Analytical column dimensions are 300 or 600 mm \times 7.5 or 7.8 mm, and

preparative columns are 60 cm \times 21.5 or 55 mm. For analysis of oligomers <3000 g/mol, a specialty column TSKgel G-Oligo-PW is available that consists of 6- μ m particles with a pore volume of 125 Å. For large polynucleotides, TSKgel G-DNA-PW is recommended which is comprised of 10- μ m particles with 4000-Å pore size.

TSKgel Super SW columns, introduced by Tosoh Bioscience, are based upon deactivated 4- μ m silica with 125- and 250-Å pore sizes making them suitable for polypeptides and proteins. The columns are of HPLC column size (30 cm \times 4.6 mm) and, therefore, also provide less solvent usage than conventional SEC. The smaller inner diameter also provides increased sensitivity in sample-mass limited situations. TSKgel Super AW columns are packed with cross-linked hydrophilic polymethacrylate particles with five pore sizes available, including a mixed bed column. Depending upon the pore size, particle size varies from 4 to 9 μ m. The standard column dimension is 150 mm \times 6.0 mm. The packings are compatible with both aqueous and polar organic mobile phases and solvent exchange is possible without swelling or shrinkage. Due to the smaller particle sizes and column dimensions, solvent consumption is about a third and separation times about half.

PSS has a range of hydrophilic packings suitable for aqueous SEC. In addition to the aforementioned PSS GRAL and GRAM packings, PSS markets a sulfonated PS-DVB gel (PSS MCX), a hydrophilic large-pore volume packing (PSS Suprema), and a packing based upon a copolymer of ethylene glycol dimethacrylate–hydroxy ethyl methacrylate (PSS HEMA). For the analysis of cationic polymers, a proprietary 10- μ m packing, PSS NOVEMA, has been introduced recently. These packings are available in a range of pore sizes and column configurations.

Biospher GM columns from Melcor Technologies (Sunnyvale, California), based upon methacrylate technology from Tessek, are recommended for the SEC separation of proteins and peptides and also can be used for non-aqueous mobile phases. Three particle sizes are available: 5 μ m for highest resolution, 10 μ m for general analytical work, and 40 μ m for semipreparative applications. Columns are available in 4- and 8 mm inner diameters and 250 or 500 mm lengths. Biospher GMB columns are useful for ultrahigh MW water-soluble biopolymers such as plasmids and viruses. The packing, based upon a hydrophilic polymethacrylate polymer, has a pore size of 10,000 Å. The hydrophilic surface eliminates nonspecific binding of proteins and lipids. Standard packed columns (stainless steel

or PEEK) are 300 \times 7.5 and 8 mm but other sizes are available upon request. Particle sizes available range from 5 to 60 μ m, which cover analytical to preparative requirements.

Thermo Electron (Waltham, Massachusetts) has introduced GFC silica-based packings (5 and 7 μ m) with three pore sizes: 150, 300, and 500 Å. A hydroxyl-bonded phase provides hydrophilic character for SEC of proteins and peptides. Thermo Hypersil BioBasic SEC columns are silica-based with a bonded hydrophilic polymer. The 5- μ m particles have pore sizes of 60, 120, 300 and 1000 Å. Analytical, preparative, and fused-silica capillary columns packed with the BioBasic SEC packings are available.

PL aquagel-OH columns (300 \times 7.5 mm) from Polymer Laboratories are packed with a macroporous, hydrophilic copolymer microparticles. Available as a range of five 8- μ m packings designated the PL aquagel-OH 30, 40, 50 and 60 with MW exclusion limits from 30,000 to 10,000,000 g/mol based upon polyoxyethylene, and a PL aquagel-OH MIXED column that has an operating range of 500–10,000,000 with respect to polyoxyethylene standards and is linear. Also available are 15- μ m versions of the PL aquagel-OH 40, 50 and 60 for high viscosity water-soluble polymers where shear degradation is a possibility. Recently, a PL aquagel-OH 20 column with an exclusion limit of 20,000 with respect to polyoxyethylene and a 5- μ m packing has been developed for high resolution analysis of low molecular weight polymers. A noted feature of all of the PL aquagel-OH columns is their excellent mechanical stability.

Waters distributes Ultrahydrogel, hydroxylated polymethacrylate-based gels available in 300 mm \times 7.8 mm columns in five pore sizes, as well as a linear column. Shodex OHPak KB-800 series, also methacrylate-based gels, come in six pore sizes packed in 300 or 500 mm \times 8 mm columns.

Macherey-Nagel (Dueren, Germany) has introduced Nucleosil 125-5 GFC, a silica-based packing that has a polyalcohol-modified surface for proteins. MICRA Gold SEC columns (Eichrom Technologies [formerly Micra Scientific], Darien Illinois), is a glycerol-bonded silica, available in six pore sizes, designed for water-soluble anionic- and neutral-water soluble polymers. Hydrocell-GFC 1500 (BioChrom Labs) is a polymeric-based column for biopolymers while Hydrocell-GPC columns are recommended for water-soluble polymers. Hypergel-AP columns from Thermo Electron, primarily designed for water-soluble polymers, are packed with 15- μ m particles to minimize the risk of shear degradation of ultrahigh MW polymers.

Nacalai Tesque (Kyoto, Japan) markets Cosmosil-5Diol-120-II (120A) and Cosmosil-5Diol-300-II columns (300 Å) that are packed with 5- μm high purity spherical porous silica deactivated with a diol-bonded phase. The Cosmosil-5-Diol-120-II can separate proteins in the range of 5000–100,000 while the Cosmosil-5-Diol-300-II can operate in the 10,000–700,000 MW range. Column dimensions of 300 and 600 mm \times 7.5 mm are available along with guard columns of 50 mm \times 7.5 mm.

Jordi Associates offers a Polar Pac WAX column, which is a PS-DVB material with a polyethyleneimine bonded phase. This material has solvent compatibility with a wide range of solvents. The columns are mainly recommended for cationic polymers but can be used for both aqueous and nonaqueous SEC. Columns are available in two sizes: 250 and 500 \times 10 mm. Columns with several pore sizes and a mixed bed for a broad MW range are available. Jordi Associates also produces hydroxylated DVB, sulfonated DVB, and glucose-modified DVB packing for aqueous SEC.

Specialty Columns

Specialty SEC columns commercially available include high-speed, high-throughput columns (≤ 10 -cm long) to monitor combinatorial polymerizations (< 5 min/sample) and small-i.d. columns (≤ 2 mm) for limited sample availability (≤ 10 - μL injection volume and microgram sample sizes) and for low flow-rate requirements, that is, SEC-MS. For example, Polymer Laboratories offers high-throughput screening columns, PL Rapide, available as mixed-bed 150 mm \times 7.5 mm and 100 mm \times 10 mm columns. Polymer Standards Service offers exceptionally high-speed columns of 50 mm \times 20 mm packed with different sorbent types and particle sizes. With these types of columns, analysis times of < 3 min are possible.

Most vendors offer high-resolution preparative SEC columns (20 mm i.d.) for a 1-mL injection volume and a 5-mg sample size for fractionation of complex formulations, preparation of polymer standards and narrow polydispersity polymer fractions. For example, Polymer Laboratories offers preparative 25-mm i.d. PS-DVB columns available in lengths of 300 and 600 mm, and 25-mm i.d. PL aquagel-OH covering a range of pore sizes. Polymer Standards Service supplies 300 and 600 mm \times 20 mm columns for PS-DVB and its complete line of aqueous packings. Waters and Showa Denko also supply preparative columns typically 300 or 500 mm \times 20 mm in size.

Low-particle shedding columns for online

light scattering detectors are available from Polymer Laboratories as part of their PLgel LS series. These packings were developed using a proprietary suspension polymerization process to eliminate nanoparticle leakage. A new packing, PSS Polar Fluorogel, has been introduced by PSS for fluorinated mobile phases, such as hexafluoroisopropanol and trifluoroethanol. This packing is available in a number of pore sizes and column dimensions.

SEC columns that can be used with different solvents without shrinking or swelling is a major advantage that offers a high degree of user flexibility. In addition to silica packings, which are indeed stable in all solvents except for basic aqueous buffers, Tosoh Bioscience has launched the TSKgel Alpha Series, which can be used with solvents ranging from water to non-polar solvents. This allows the same column to be used for both aqueous and non-aqueous size separations.

In addition to using SEC for MWD measurements, SEC also is employed for desalting biopolymer solutions and cleaning up complex biological samples in which low MW compounds of interest are separated from unwanted high MW materials. To help meet these needs, Showa Denko has introduced Shodex MsPak GF-310 poly(vinyl alcohol)-based SEC columns for the removal of high MW species when analyzing drugs in serum and urine. For SEC sample cleanup, a series of Shodex CLNpak porous polymer columns has been introduced to remove water and high MW compounds, such as lipids, polymers, and pigments from a wide range of food and environmental samples. Waters' Envirogel GPC cleanup columns (150 or 300 mm \times 19 mm) and Polymer Laboratories' PL EnviroPrep columns (150 or 300 \times 25 mm) are designed to remove high MW interfering materials, such as lipids from environmental samples.

The use of DNA-wrapped carbon nanotubes has great potential in medical, microbiological, and environmental science research. By wrapping one strand of DNA around the surface of a carbon nanotube, researchers can create a sensor that is targeted for a particular piece of complementary DNA. A new SEC packing material has been developed by Sepax Technologies (Newark, DE) called CNT that can separate carbon nanotubes, nanoparticles, and nanorods based upon their lengths. The columns are packed with spherical silica particles of 5-, 7- and 10- μm diameters with 300-, 500-, 1000- and 2000-Å pore sizes with a proprietary bonded phase. The recovery for the length-dependent separation of DNA-wrapped carbon nanotubes is nearly 100%.

Future Challenges and Needs

SEC column technology has followed closely behind developments with interactive HPLC columns, and, as such, has reached a fairly mature level. Nevertheless, column improvements are still needed for increased column resolution, noninteractive packing surface chemistries for water-soluble polymers, and higher temperature stability.

Future challenges include the development of high-resolution packings to handle ultra high MW polymers and column hardware designed to reduce high elongational strain rates, a major contributing factor to polymer shear degradation (4). One of the difficulties in developing large-pore size packings with narrow pore-size distributions is packing fragility. The use of larger size packings to reduce shear degradation unfortunately leads to reduced column efficiency. Decreased efficiency is even more detrimental for ultrahigh MW polymers because of their already extremely low diffusion coefficients. Combinatorial polymerization reactions are becoming more popular, thus, very short SEC columns for high-sample throughput (< 5 min/sample), as demonstrated by PSS, is indeed a major advancement.

With the growing popularity of LC-MS, mainly for biologicals as well as for lower MW synthetic polymers, there is more interest in using small i.d. SEC columns to meet low-flow rate requirements for on-line SEC-MS, especially with limited sample availability. Although SEC column resolution is compromised because of the low column pore volume, the exceptionally high MS resolution and selectivity enhances the overall SEC-MS resolution. Thus, small-i.d. SEC columns for online MS offer important advantages, provided that the polymer can be ionized with minimum fragmentation and multiple charging.

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