High-Performance SEC Column Technology

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

Introduction

The purpose of this paper is to review desired characteristics of size exclusion chromatographic (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 average pore size and packing geometry. Figure 1 shows a typical SEC calibration curve in which log MW (molecular weight) 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 elute 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 elutes later. Smaller molecules freely diffuse into and out of the packing sampling both the column pore volume and interstitial volume, and thus elute 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 MWD (molecular weight distribution) can be determined from a calibration curve or using a MW-sensitive detector.

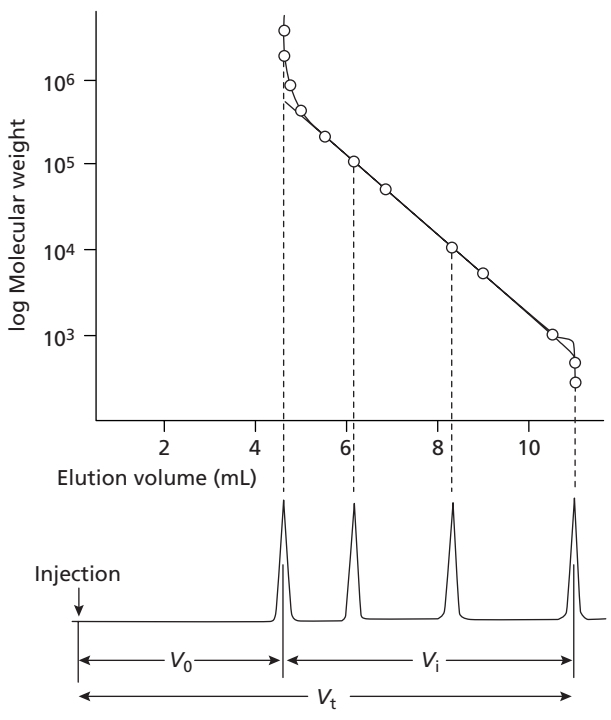
Desired SEC Column Characteristics

Because of the difference in separation mechanism between SEC and interactive HPLC, in which 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 (Table 1). Desired characteristics in SEC column technology are high chromatographic resolution (efficiency and selectivity), broad molecular weight separation range, calibration curve (log MW vs elution volume) linearity, and packing inertness and ruggedness.

It is important to realize that a polymer sample elutes 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 MW accuracy and precision. A discussed below, 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 (D_2) of the calibration curve (log MW vs V_r) must be minimized. In other words, for increased resolution, the elution volume required to separate two polymers that have by a 10-fold 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

Figure 1: A typical SEC calibration curve.



longer or more columns in series is a more practical approach for decreasing the calibration curve slope.

Also for optimum SEC resolution, both peak broadening and slope must be minimized. SEC columns that have high plate numbers may 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 ≤5 µm packings in ≥25 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 accurate MW data. For high SEC column efficiency, small particles are needed, as in the instance 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 range from 3 to 20 µm, 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 laboratories 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 encompass 3–5 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/or poly(divinylbenzene) (DVB) are commonly used with a wide range of organic solvents, such as THF, toluene, chloroform, DMF, DMAC, DMSO, *N*-methylpyrrolidinone, HFIP and TCB. Hydrophilic polymeric packings, such as poly(vinyl alcohol), poly(acrylamide), or sulphonated polystyrene, find use with water-soluble polymers. Silica-based packings, either bare or silanized, can be used for the analysis of organosoluble polymers, while hydrophilically modified silica is used extensively for biopolymers and synthetic water-soluble polymers. For SEC of water-soluble polymers, however, there is no universal SEC system. Column/mobile phase conditions depend upon the chemical structure and ionic nature of the polymer. Selection is typically performed empirically by adjusting and fine-tuning pH, ionic strength, ratio of aqueous/organic solvent and temperature to prevent packing interactions and unwanted intramolecular electrostatic interactions.[5]

In general, silica-based packings are more versatile than polymeric packings because they 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 analysing basic polymers. Finally, 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.

New Packings for Organosoluble Polymers

The smallest PS-DVB packing particle size commercially available is 3 µm. These packings are limited to the SEC analysis of lower MW polymers because of the potential of shear degradation with high MW polymers. Several manufacturers have introduced SEC columns of dimensions that are smaller than conventional columns that typically range from 7–8 mm i.d. and 250–300 mm in length. Advantages of reduced column dimensions are faster analysis times, higher sample throughput, 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, resolution and MW accuracy can be compromised with reduced column efficiency and pore volume.

Showa Denko (Kawasaki, Japan) Series K-800 (8 mm i.d. × 300 mm) are available in up to eight different pore sizes and linear columns and are available packed in THF, chloroform, DMF, toluene or HFIP. Shodex GPC KF-400, K-400 and HFIP-400 columns are now available in 4.6 mm × 300 mm packed with a 8 µm PS-DVB. Column dimensions of Shodex GPC LF series, packed with PS-DVB 8 µm particles, are 4.6 mm i.d. × 250 mm for high-resolution separations and 6.0 mm i.d. × 150 mm for rapid analysis. For SEC of more crystalline polymers, such as polyamides and polyesters, the mobile phase of choice is hexafluoroisopropanol (HFIP). To meet this need, Showa Denko has introduced PS-DVB packings (Shodex HFIP-600 series) that are packed in HFIP, also available with 3 µm particles packed in 150 mm × 6 mm i.d. columns.

The Shodex GPC KF-600 series consist of 3 µm packings in nine different pore sizes from 20 to >1000 Å in 150 mm ×

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)

6 mm i.d. columns. This shorter, narrower i.d. column requires about one third of the solvent usage and half the analysis time compared with 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 µm, 8.0 mm i.d. × 300 mm) is designed to have a wide MW linear range and improved lower molecular weight linearity.

PS/DVB packings are also available from TosoHaas (Tosoh Corp., Tokyo, Japan) that supplies eight pore sizes and two-mixed beds. Depending upon pore size, packings are 5, 6, 9, 10 and 13 µm packed in 7.5 or 7.8 mm i.d. × 300 or 600 mm columns, as well as preparative 21.5 mm i.d. × 600 columns. A high-temperature version, TSKgel H-HT rated for 140 °C, is also available. A rather interesting approach to "mixed-bed" column technology is the TSKgel Multipore HxL-M packing. This packing is designed with multiple pore sizes within a single particle. Other companies provide similar capabilities by using a mixed-bed approach in which different pore size particles are blended in a single column. The TosoHaas Multipore approach apparently gives better linearity over a wide MW range.

TosoHaas new generation TSKgel Super HZ are high-performance SEC packings based on small-particle technology. The HZ products for non-aqueous SEC are packed with 3 µm PS-DVB particles in 4.6 mm × 150 mm and 6.0 mm × 150 mm columns with five different pore sizes. The smaller pore sizes can also separate monomers, oligomers and polymer additives. TSK-gel H_{HR} line consists of 12 new columns (300 × 7.8 mm i.d.) with a wide variety of pore sizes including four mixed bed columns. The significant feature of the H_{HR} columns is their tolerance to different solvents with minimal swelling. Some of the common mobile phases that can be used are THF, chloroform, dichloromethane, DMF, DMSO, HFIP, acetone, ethanol and o-dichlorobenzene.

Polymer Laboratories (Church Stretton, Shropshire, UK) has five types of mixed bed columns packed with 5, 10 or 20 µm PS/DVB particles covering from two to five decades of MW separation. Their 20 mm packing has an exclusion limit of 40×10^6, and is specifically designed for the analysis of ultrahigh MW polymers. PS/DVB columns of 4.6 mm i.d. × 250 mm, available in different particle sizes, have reduced solvent consumption. These columns have high-temperature stability up to 220 °C. Polymer Laboratories also has PL HFIPgel columns packed in HFIP available in 4.6 mm i.d. × 250 mm and 7.5 mm i.d. × 300 mm columns. Oligopore columns, which have significantly increased pore volume, have high resolution for oligomers up to about 4500 MW.

Polymer Standards Services (Mainz, Germany) offers PS/DVB packings (PSS SDV) from 5 to 20 µm in a range of pore sizes, column dimensions and as mixed-bed columns. Polymer Standards Services offers several 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 up to 220 °C.

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

Jordi Associates (Bellingham, Massachusetts, USA) SEC packings are prepared from DVB, rather than PS/DVB. DVB packings are claimed to have greater stability and larger pore volumes. These 5 µm packings are available in five pore sizes, as well as a mixed bed, and in different column configurations. BioChrom Labs (Terre Haute, Indiana, USA) has introduced Hydrocell GPC 3000 HS, a PS-DVB packing of 7–13 µm available in columns of 7.8 mm i.d. × 150 mm and 300 mm.

New Packings for Biopolymers and Water-Soluble Polymers

TosoHaas produces silica-based (TSKgel SW) and polymeric-based (TSKgel PW) packings. TSKgel SW is available in three pore sizes (125, 250 and 450 Å) and 5, 8, 10, 13 and 17 µm particle size. Column dimensions are 7.5 or 7.8 mm i.d. × 15, 30 and 60 cm, as well as 21.5 and 55 mm i.d. × 30 and 60 cm for preparative separations. TSKgel PW packings are prepared via copolymerization of ethylene glycol and methacrylate. Pore sizes are <100, 125, <200, 200, 500, 1000 and >1000 Å, and particle sizes range from 6 to 25 µm. Analytical column dimensions are 7.5 or 7.8 mm × 300 or 600 mm, and preparative columns are 21.5 or 55 mm i.d. × 60 cm. For analysis of oligomers <3000 g/mol, a speciality column TSKgel G-Oligo-PW is available that consists of 6 µm particles with a pore volume of 125 Å. For large polynucleotides, TSKgel G-DNA-PW is recommended which is comprised of 10 µm particles with 4000 Å pore size.

TSKgel Super SW columns, introduced by TosoHaas, is based on deactivated 4 µm silica with 125 and 250 Å pore size making them suitable for polypeptides and proteins. The columns are of HPLC column size (4.6 mm × 30 cm) and therefore also provide less solvent usage than conventional SEC. The smaller i.d. 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 on the pore size, particle size varies from 4 to 9 µm. The standard column dimension is 6.0 × 150 mm. The packings are compatible with both aqueous and polar organic mobile phases and solvent exchange is possible without swelling or shrinkage. Because of the smaller particle sizes and column dimensions, solvent consumption is about a third and separation times about half.

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

Biospher GM columns from Melcor Technologies (Sunnyvale, California, USA), based on methacrylate

technology from Tessek, are recommended for the SEC separation of proteins and peptides and can also be used for non-aqueous mobile phases. Three particle sizes are available: 5 µm for highest resolution, 10 µm for general analytical work and 40 µm for semi-preparative applications. Columns are available in 4 and 8 mm i.d. and 250 or 500 mm in length. Biospher GMB columns are useful for ultrahigh MW water-soluble biopolymers, such as plasmids and viruses. The packing, based on a hydrophilic polymethacrylate polymer, has a pore size of 10 000 Å. The hydrophilic surface eliminates non-specific binding of proteins and lipids. Standard packed columns (stainless steel or PEEK) are 300 × 7.5 and 8 mm i.d. but other sizes are available upon request. Particle sizes available range from 5 to 60 µm, which cover analytical to preparative requirements.

Thermo Electron (Bellefonte, Pennsylvania, USA) has introduced GFS silica-based packings (5 and 7 µm) with three different pore sizes: 150, 300 and 500 Å. A hydroxyl-bonded phase provides hydrophilic character for SEC of proteins and peptides. BioBasic SEC columns are silica-based with a bonded hydrophilic polymer. The 5 µm particles have pore sizes of 60, 120, 300 and 1000 Å. Analytical, preparative and fused-silica capillaries packed with the BioBasic SEC packings are available.

PL Aquagel-OH columns (300 × 7.5 mm i.d.) from Polymer Laboratories are packed with a macroporous, hydrophilic copolymer microparticles. Available are 8 µm packings with MW exclusion limit of 35 000 based on polyoxyethylene (PEO) and a PL-Aquagel-OH Mixed column that has an operating range of 500–10 000 000 with respect to PEO standards. Also available is a 15 µm version with pore diameters of 4000, 5000 and 6000 Å for ultrahigh MW water-soluble polymers up to 20 000 000 based on PEO. 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 7.8 mm i.d. × 300 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 8 mm i.d. x 300 or 500 mm columns.

Macherey-Nagel (Düren, Germany) has introduced Nucleosil 125-5 GFC, a silica-based packings that has a polyalcohol-modified surface for proteins. MICRA Gold SEC columns (Eichrom Technologies, formerly Micra Scientific), is a glycerol-bonded silica, available in six different 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 µm particles to minimize the risk of shear degradation of ultrahigh MW polymers.

Nacalai Tesque (Kyoto, Japan) markets Cosmosil-5Diol-120-II (120 Å) 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 5000–100000 while the Cosmosil-5-Diol-300-II can operates in the 10000–700000 MW range. Column dimensions of 7.5 mm i.d. × 300 and 600 mm lengths are available along with guard columns of 7.5 mm × 50 mm lengths.

Jordi Associates offers a Polar Pac WAX column, which is a poly(divinylbenzene)-base 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 non-aqueous SEC. Columns are available in two lengths: 250 and 500 × 10 mm i.d. Columns with several different pore sizes and a mixed bed for a broad MW range are available. Jordi Associates also produces hydroxylated DVB, sulphonated DVB and glucose-modified DVB packings for aqueous SEC.

Speciality Columns

Speciality 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 i.d.) for limited sample availability (≤10 µL injection volume and mg sample size) and for low flow-rate requirements (i.e., SEC–MS). For example, Polymer Laboratories offers high-throughput screening columns, PLgel HTS, available as mixed-bed 7.5 mm i.d. × 150 mm columns. Polymer Standards Service offers exceptionally high-speed columns of 20 mm i.d. × 50 mm lengths 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 >1 mL injection volume and >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 Standard Services supplies 20 mm i.d. × 300 and 600 mm columns for PS/DVB and its complete line of aqueous packings. Waters and Showdex also supply preparative columns, typically 20 mm i.d. × 300 or 500 mm in length.

Low-particle shedding columns for online light scattering detectors are available from Polymer Laboratories as part of its 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 HFIP and trifluoroethanol. This packing is available in several pore sizes and column dimensions.

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

In addition to using SEC for MWD measurements, SEC is also 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 analysing drugs in serum and urine. For SEC sample clean-up, a series of Shodex CLNpak porous polymer columns have 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 clean-up

columns (19 mm i.d. × 150 or 300 mm in length) are designed to remove high-MW interfering materials, such as lipids from environmental samples.

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, non-interactive packing surface chemistries for water-soluble polymers and higher temperature stability.

Future challenges include the development of high-resolution packings to handle ultrahigh 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, which is even more detrimental for ultrahigh MW polymers because of their already extremely low diffusion coefficients.

Combinatorial polymerization reactions are becoming more popular; thus the use of very short SEC columns for high-sample throughput (<5 min/sample), as demonstrated by Polymer Standards Services, is indeed a major advance.

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 on-line MS offers important advantages, provided that the polymer can be ionized with minimum fragmentation and multiple charging.

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