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Research Article

Ultra-high performance size-exclusion chromatography of synthetic polymers

Demonstration of capability

Ultra-high performance size-exclusion chromatography (UHP SEC) is a newly developed disruptive technology that allows the high-resolution separation of synthetic polymers in as little as 2 min. The capability of UHP SEC for the characterization of synthetic polymers in organic solvents has been demonstrated. Using the Waters ACQUITY UPLC[®] H-Class system and ethylene-bridged hybrid size-exclusion chromatography (SEC) columns packed with 1.7 to 2.5- μ m particles with pore sizes ranging from 45 to 900 Å, size-based separations of polystyrene and poly(methyl methacrylate) standards in tetrahydrofuran and poly(ethylene oxide) standards in 20 mM ammonium acetate in methanol are achieved within 2–4 min. The speed of analysis is about ten times faster than conventional SEC separations, and greater resolution is achieved. Average molecular weights of selected commercial polymers have been determined using ultra-high performance and conventional SEC. Average *M* data of analyzed samples are in good agreement using the two approaches. An inherent limitation of SEC in UHP mode is the characterization of very high *M* polymers (above ca. 2 million Da) due to the deformation and/or mechanical shearing of large molecules at high flow rates.

Keywords: Polystyrene / Poly(methyl methacrylate) / Poly(ethylene oxide) / Ultra-high performance size-exclusion chromatography / UHP SEC
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1 Introduction

Size-exclusion chromatography (SEC) [1–4] is the preferred method for the determination of the molecular weight parameters of both natural and synthetic polymers due to its universality, good accuracy, reliability, reproducibility, and low sample consumption. The technique of SEC was first described by Moore [5], in which porous particles of styrene-divinylbenzene were synthesized and packed into chromatographic columns. This work led to the development of the

Waters Styragel[®] family of products. To this day, styrene-divinylbenzene gel particles remain the most widely used chromatographic media for the size-based characterization of synthetic polymers.

However, the poor mechanical strength of these particles has limited the advancement of this technique. Large particles are required with low linear velocities to mitigate the shear-induced compression and fracturing of the media. In addition, large column diameters are utilized to minimize the additional extra-column bandspreading resulting from subideal chromatographic instrumentation. To achieve sufficient resolution of separated polymeric species, a column set composed of at least two—or more often three—analytical columns (300 × 7.5 mm id) is used. Typical flow rates are 1–2 mL/min in order to not damage the packed column bed, resulting in run times of 30–60 min.

A substantial acceleration of SEC separations remains difficult to achieve due to mechanical stability limitations of conventional SEC stationary phases. Barth and Saunders reviewed some of the basics of SEC and looked at the current status of column technology [6] including developments for faster and higher resolution size separations [7]. Several approaches including higher flow rates using conventional columns [8], shorter length of analytical columns [9], shorter

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Abbreviations: BEH, ethylene-bridged hybrid; ELS, evaporative light scattering; MeOH, methanol; MPD, mean pore diameter; NH₄Ac, ammonium acetate; PDA, photodiode array; PEO, polyethylene oxide; PMMA, polymethylmethacrylate; PS, polystyrene; RI, refractive index; SPV, specific pore volume; THF, tetrahydrofuran; TMS, trimethylsilyl; SEC, size-exclusion chromatography; UHP, ultra-high performance; UHP SEC, ultra-high performance size-exclusion chromatography; UPLC, ultra-performance LC

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and wider diameter columns (<http://www.polymer.de/products/columns-for-gpcsec-gpcsec-high-speed-columns.html>), and overlaid injections [10–12] are promoted to speed up SEC separations. However, there are tradeoffs to each of these approaches. The first three approaches result in a loss of chromatographic resolution, while the last adds complexity to the chromatographic software and instrumentation.

In addition to the mechanical stability limitations, traditional polymeric resins used for SEC also shrink and/or swell in different solvents; this limits the ability to switch mobile phases for a given column. Ethylene-bridged hybrid (BEH) particle technology, patented by Waters [13–15], does not suffer from either of these limitations. Columns packed with sub-2 μm BEH particles have been used successfully for several separation modes, including RP, hydrophilic interaction, and SEC [16, 17]. Uliyanchenko *et al.* [18] used ultra-performance LC (UPLC) technology to explore the size-based separation of polymers using UPLC columns. However, the low pore volume of the BEH particles (0.7 cc/g) resulted in suboptimal selectivity for polymer separations over a wide range of molar masses.

The wide acceptance of ultra-high performance (UHP) SEC technology will be highly dependent on the availability of packing materials with appropriate pore sizes to cover a wide range of molar masses of analyzed polymers. Recently, Waters succeeded in the preparation of sub-2 μm BEH packing materials that are optimized for the SEC analysis of proteins and their aggregates [19]. These materials have about 75% higher pore volume than the original BEH materials. In this work, UHP SEC separations of synthetic polymers using prototypes of BEH SEC columns are demonstrated for molar masses up to about 2 000 000 Da.

2 Materials and methods

2.1 Materials

Narrow polystyrene (PS), poly(methyl methacrylate) (PMMA), and polyethylene oxide (PEO) standards commercially available from Agilent (Santa Clara, CA, USA) were used to demonstrate the feasibility of UHP SEC separations. Several individual PS standards were used with M_p of 162, 580, 761, 1060, 1320, 1700, 2450, 3250, 5050, 7000, 9200, 11 600, 22 000, 34 500, 44 200, 66 300, 70 600, 120 000, 156 000, 200 000, 310 000, 560 000, 1 250 000, 2 750 000, and 5 000 000 g/mol. In addition, PS mixtures labeled as PS2 Mix A and PS2 Mix B were used to calibrate each individual BEH SEC column. In the case of PMMA and PEO standards, mixtures labeled as red, yellow, and green were used. In addition, selected samples of commercially available polymer samples labeled as polymers A–D were also analyzed.

Tetrahydrofuran (THF), methanol (MeOH), and ammonium acetate (NH_4Ac) were purchased from Fisher Scientific (Fairlawn, NJ, USA). 20 mM NH_4Ac was prepared by adding the appropriate amount of salt to MeOH.

Both narrow standards and selected samples were prepared in SEC eluent at concentrations of about 0.5–1.0 mg/mL per component. Samples were allowed to dissolve in a mechanical shaker overnight. Polymer solutions of narrow standards were used as is, while solutions of commercial polymers were filtered through Whatman 0.45 μm PTFE filters prior to analysis.

2.2 Experimental setup

The instrument used for SEC separations was a Waters ACQUITY UPLC[®] H-Class system with quaternary solvent and sample manager, and column module. The system was configured using hexane/THF compatibility kit (Waters) to allow use of organic solvents. The sample manager was equipped with a flow-through needle injector. Prototype 4.6 $\times$ 150 mm SEC columns were obtained from Waters. They were packed with 1.7 to 2.5 μm particles of varying pore sizes. For comparison, an Agilent 4.6 $\times$ 150 mm PLgel MiniMix D column was used.

The mobile phase consisted of THF or MeOH with 20 mM NH_4Ac . A flow rate of 1 mL/min was used. Under these conditions, BEH SEC columns packed with 1.7 μm particles generate backpressure of approximately 5000 psi. Injection volumes were 2, 5, or 10 μL , depending on the number of columns in series. All separations were performed at ambient temperature. UV detection was performed with the ACQUITY PDA detector, monitored at 254 nm. Acquisition rate was 10 points/s, with the time constant off. Evaporative light scattering (ELS) detection was performed with the ACQUITY ELS detector. Settings were as follows: gas pressure: 40.0 psi; data rate: 10 points/s; gain: 500; time constant: off; nebulizer mode: cooling; drift tube temperature set point: 50°C. UHP SEC system control, data collection, and processing were performed using Waters Empower software v. 2.0.

2.3 UHP SEC columns

Columns packed with prototype chromatographic media of different pore size and surface chemistry were provided by Waters. The media were characterized as follows: the specific surface areas, specific pore volumes (SPVs), and mean pore diameters (MPDs) were measured by the multipoint nitrogen sorption method using a Micromeritics Instruments (Norcross, GA, USA) ASAP 2405. The specific surface area was calculated using the Brunauer–Emmett–Teller method, the SPV was determined at a single point for $P/P_0 \geq 0.98$, and the MPD was calculated from the desorption leg of the nitrogen isotherm using the Barrett–Joyner–Halenda method. For particles with $\text{MPD} \geq 400 \text{ \AA}$, the SPV and MPD were determined by mercury porosimetry using a Micromeritics AutoPore IV. Particle-size analyses were carried out using a Beckman Coulter Multisizer 3 analyzer (30 μm aperture, 70 000 counts).

Table 1. Characteristics of the SEC media prior to bonding

Column id	MPD (Å)	SPV (cm ³ /g)	SSA (m ² /g)	Mean d_p (μm)	Bonded phase
XBridge TM (Ma) C ₁₈	130	0.7	NA	10	C ₁₈
XBridge TM C ₁₈	130	0.7	NA	5	C ₁₈
XBridge TM C ₁₈	130	0.7	NA	3.5	C ₁₈
ACQUITY [®] BEH C ₁₈	130	0.7	NA	1.7	C ₁₈
BEH 45	46	0.75	596	1.71	Unbonded
BEH 90	84	1.32	613	1.65	Unbonded
BEH 125	126	1.39	393	1.54	Unbonded
BEH 200	210	1.31	217	1.90	Unbonded
BEH 450	435	1.18	81	1.81	Unbonded
BEH 900	905	0.82	30	2.17	Unbonded
BEH 125 Diol	126	1.39	393	1.54	Diol
BEH 200 Diol	212	1.36	227	1.70	Diol
BEH 450 Diol	432	1.10	82	1.47	Diol
BEH 45 TMS	46	0.75	596	2.50	TMS
BEH 125 TMS	125	1.29	394	2.41	TMS
BEH 200 TMS	210	1.31	217	1.90	TMS
BEH 450 TMS	442	1.14	80	2.10	TMS

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Columns of 4.6 × 150 mm were packed with 1.7 to 2.5 μm BEH particles with different surface modifications and mean pore sizes. Surface modification comprised unbonded, diol-bonded, and trimethylsilyl (TMS)-bonded particles. Pore sizes ranged from 45 to 900 Å. The materials used in this work are summarized in Table 1. To demonstrate the effect of particle size on the width of polymer peak and determined *M* data, HPLC columns packed with C₁₈ particles with pore size 130 Å and varying particle sizes (1.7, 3.5, 5, and 10 μm) were also utilized.

3 Results and discussion

3.1 Effect of particle size

For more than four decades, reducing the stationary-phase particle size has been exploited to improve separation efficiency. Until recently, this approach reached a limit at which the benefits of further reduction of particle size could not be fully realized because of pressure and bandspreading limits of the chromatographic system resulting from the columns and the LC instrumentation.

Within the chromatographic system, both column and extra-column sources contribute to bandspreading of the chromatographic peak. Sources of extra-column bandspreading include the injector, the fluidic path between the injector and the column, and from the exit of the column to the detector including the flow cell. To minimize the extra-column broadening, separations were carried out on the low dispersion Waters ACQUITY UPLC[®] H-Class system (extra-column dispersion of 10–12 μL, measured at 4.4% peak height) [20].

Column contributions to bandspreading are described by the van Deemter equation [21], which is shown in modified form below:

$$H = a \cdot d_p + b \frac{D_m}{u} + c \frac{d_p^2 u}{D_m} \quad (1)$$

where *H* is the height equivalent of a theoretical plate, *a*, *b*, and *c* are reduced constants, *d_p* is the mean particle size, *D_m* is the analyte diffusion coefficient, and *u* is the linear velocity. From this equation, one sees that *H* depends on the particle size of the packing material, the analyte diffusivity, and the mobile-phase velocity. In the case of polymer separations, the linear velocity is typically much higher than the optimum and in combination with the low diffusion coefficient of polymers, the second *b* term in the above equation becomes negligible. At high linear velocities, the third *c* term is proportional to *d_p*². Under these conditions, substantial gains in efficiency and speed can be achieved when using smaller particles.

For the case of polymer separations by SEC, dispersity *Đ* [22], of the sample also impacts peak width. As molecular weight increases, the difference in retention time between a polymer of *n* units in length from one of *n* + 1 units in length decreases. For polymers with a molecular weight above a few thousand, SEC does not have the resolving power to fully separate an *n*-mer from an (*n* + 1)-mer. But the slight difference in retention time between the polymers varying by single monomer units results in a single broad peak whose breadth depends on the analyte dispersity. Thus, the width of the observed chromatographic peak depends on the system dispersion, the chromatographic dispersion, and the sample polydispersity.

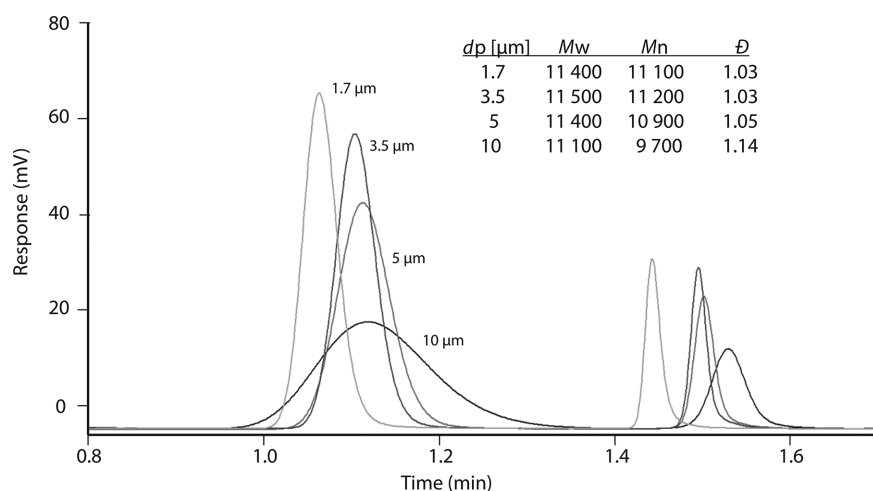


Figure 1. Chromatograms of PS standard ($M_p \sim 11\,600$ g/mol, $\bar{D} \sim 1.03$) obtained on XBridgeTM C₁₈ and ACQUITY[®] C₁₈ columns (4.6×150 mm) packed with different size particles: 10, 5, 3.5, and 1.7 μm . Mobile phase, THF; flow rate, 1 mL/min; detection, UV at 254 nm.

The advantage of utilizing smaller particles for polymer separation in SEC mode is demonstrated in Fig. 1. It shows an overlay of chromatograms of an 11 600 g/mol PS standard with a reported $\bar{D}$ of 1.03, run in SEC mode on columns packed with C₁₈ particles with particle sizes ranging from 1.7 to 10 μm . The SEC linear range for PS on this column is from about 3000 to 80 000 Da. The results show that as the particle size decreases, the calculated dispersity of the analyzed polymer correspondingly becomes closer to the reported $\bar{D}$ value, and thus provides greater accuracy of M data.

The utility of smaller particles is most evident in the characterization of oligomers. The combination of shorter mass-transfer path and high linear velocities, using a column of appropriate pore size distribution enables fast, high-resolution separations in this lower M_w range. Figure 2 shows a separation of low M PS standards obtained on a column set that comprised two UHP SEC columns (4.6×150 mm each) connected in series and packed with 2 μm particles with a 45 Å mean pore size. The separation of individual mers up to about a ten-mer can be achieved in 3.5 min.

3.2 Effect of pore size

The thermodynamic retention in SEC is the fraction of intraparticle pore volume that is accessible to the analyte:

$$K_D = \frac{V_R - V_0}{V_i} \quad (2)$$

where K_D is the SEC partition coefficient, and V_R , V_0 , and V_i are the respective retention volumes of the analyte of interest, the interstitial volume, and the intraparticle volume. The exclusion of an analyte depends on both the properties of the chromatographic support as well as the size and geometry of the analyte. K_D is a measure of the extent that an analyte can penetrate into the pore volume of the column.

Selectivity in SEC depends on the pore size distribution. Selectivity can be characterized by the slope of the line in the linear portion of the calibration curve, which can be defined by the relationship:

$$\log M = m \cdot K_D + b \quad (3)$$

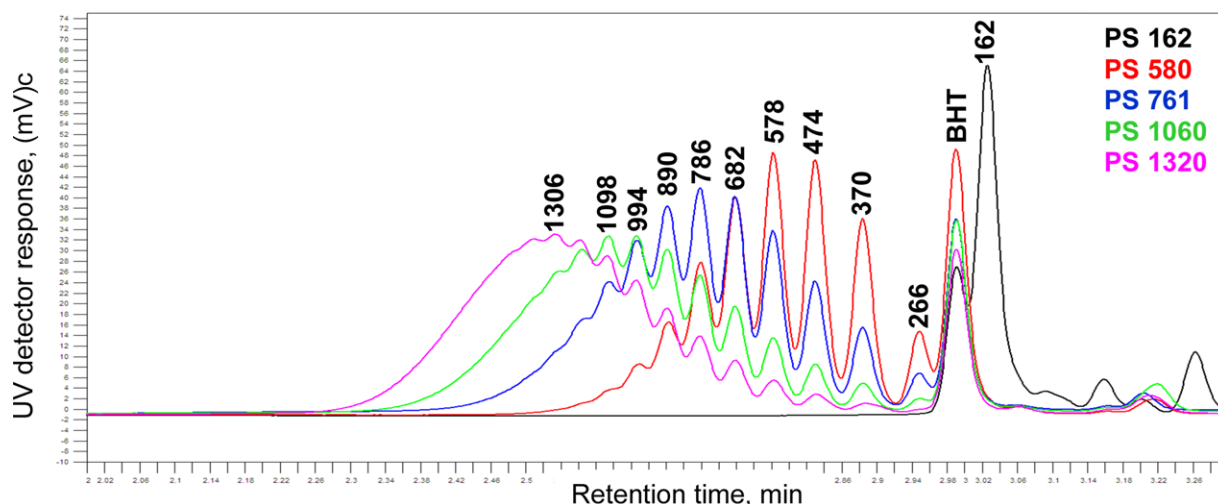


Figure 2. Overlay of chromatograms of low M PS standards with M_p 162, 580, 761, 1060, and 1320 obtained on two 4.6×150 mm columns connected in series containing unbonded BEH 45 Å particles. Mobile phase, THF; flow rate, 1 mL/min; detection, UV at 254 nm.

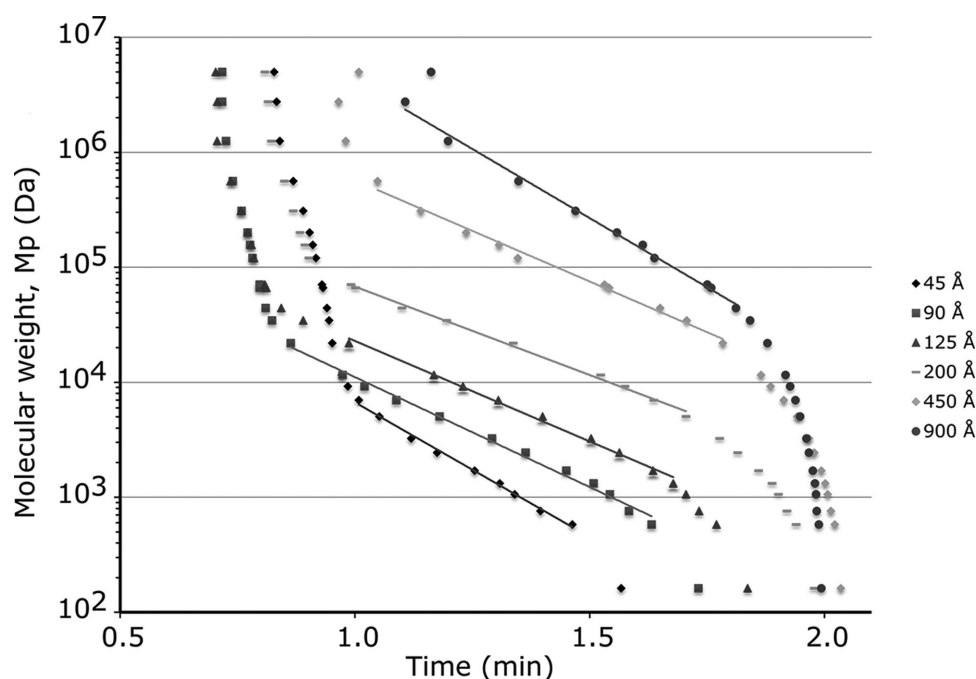


Figure 3. Overlay of calibration curves (first-order fit) of narrow PS standards obtained on BEH unbonded columns (4.6×150 mm) with different pore sizes. Mobile phase, THF; flow rate, 1 mL/min; detection, ELS.

where m and b are the respective slope and intercept of the line. The slope depends on the intraparticle pore volume as well as the breadth of the pore size distribution. A shallower slope reflects a narrower pore size distribution and greater ability to separate analytes that are similar in size. Higher selectivity translates into greater precision and accuracy in the determination of M and $\bar{D}$. However, as selectivity increases, the molecular weight dynamic range correspondingly decreases.

High-pore-volume BEH particles of different pore size (45–900 Å) and surface chemistry (unbonded, diol, and TMS) were packed into 4.6×150 mm columns. The properties of each of the materials tested are given in Table 1.

The overlay of calibration curves of prototype unbonded UHP SEC columns is depicted in Fig. 3. The curves used a first-order fit to show the linear separation range. These columns cover the separation range of molar masses of PS standards from about monomer (162 g/mol) to over 2 million (2 750 000) Da. The slopes of the calibration curves of the columns packed with BEH particles with 45 and 900 Å pore sizes are steeper than that for columns packed with particles with pore sizes of 90, 125, 200, and 450 Å. This is attributed to different synthesis routes for making these particles, resulting in a lower pore volume of the first two materials, as shown in Table 1.

3.3 Effect of surface chemistry

SEC-based separations require that analytes do not adsorb to the stationary phase surface. Enthalpic interactions can be minimized by proper selection of both the SEC column packing and eluent type for the polymer of interest. The BEH particle is created by the polymerization of tetraethoxysilane with

bis(triethoxysilyl)ethane. The resulting particle contains surface silanols that are capable of interaction with hydrophilic functional groups under certain conditions. An example in Fig. 4 shows the impact of surface chemistry on the retention of two proprietary polymers. Polymer A has a hydroxyl functional group and polymer B has an epoxy functional group. Separations were performed either on a column containing unbonded particles or on one containing TMS-bonded particles. In both instances, THF was used as the mobile phase. The chromatograms demonstrate that for these particular polymers, elution volume on the column with TMS-bonded particles was substantially less than that on one with unbonded particles. The TMS surface modification significantly reduces the available silanols for interaction, and in these instances the TMS bonding mitigates the adsorptive properties of the surface silanols on the BEH particle. To date, we have found that the TMS-bonded materials showed minimal interactions with polymers/solutes tested when using THF mobile phase. Separations on columns packed with unbonded particles were found to exhibit unwanted adsorption for some hydrophilic polymers with THF mobile phase.

3.4 Applications

In this work, low-dispersion UV and ELS detectors were employed. Figure 5A and B shows the separation of PS standards on a prototype diol column with 200 Å pores. The separation of eight components is achieved within 2 min. Excellent repeatability is demonstrated using both detectors. For comparison, the same separation was performed using a 5 μ m PLgel MiniMix D column on the low dispersion ACQUITY instrument (Fig. 5C). While there are selectivity differences between the two different columns, the

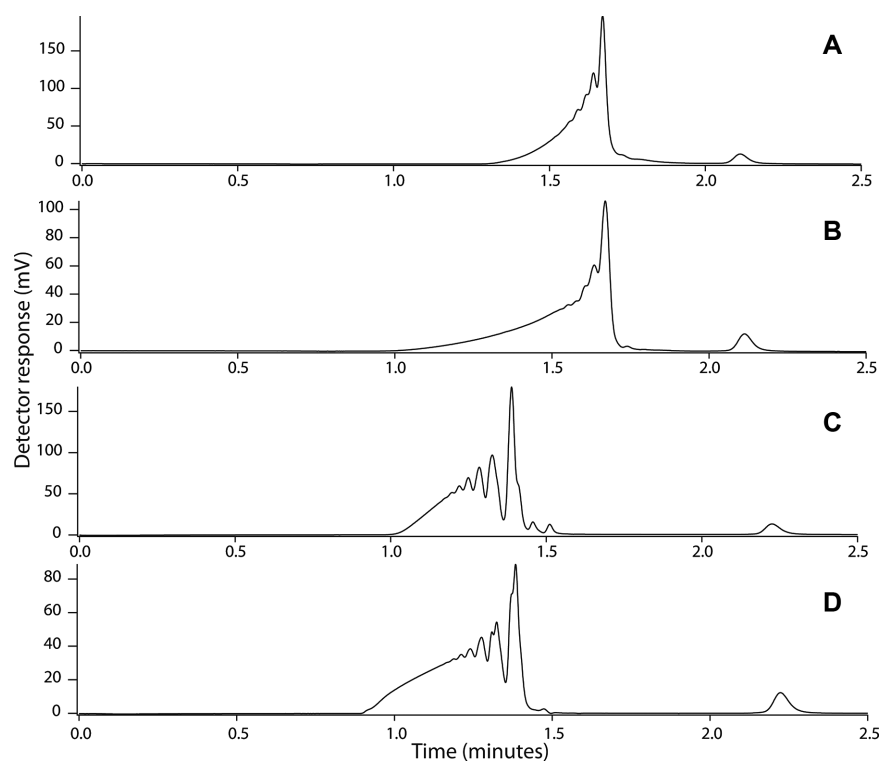


Figure 4. Stacked chromatograms of SEC separation of two proprietary polymers on BEH 45 unbonded and BEH 45 TMS columns, 4.6×150 mm in THF at 1 mL/min using UV detection at 254 nm. (A) Polymer A on BEH 45 unbonded; (B) polymer B on BEH 45 unbonded; (C) polymer A on BEH 45 TMS; (D) polymer B on BEH 45 TMS.

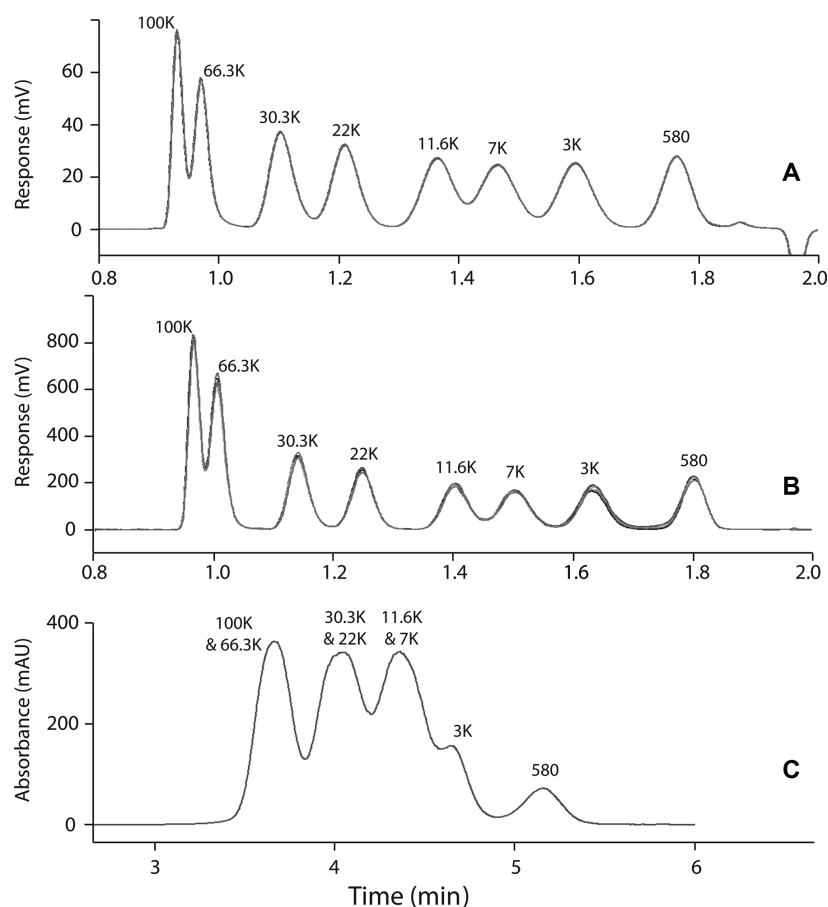


Figure 5. Chromatograms of PS standards on (A) BEH 200 diol, 4.6×150 mm, detection, UV at 254 nm; (B) same as (A), but with ELS detection. Five replicate injections are shown, demonstrating repeatability; (C) $5 \mu\text{m}$ PLgel MiniMix D (4.6×250 mm); detection, UV at 254 nm. In all cases, mobile phase was THF and flow rate was 1 mL/min.

improved resolution obtained on the prototype column is attributed primarily to the significantly higher plate count (45 000 theoretical plates/column) achievable with the smaller particles.

When two columns of different pore sizes are connected in series, the dynamic range of separation can be extended. Figure 6A shows the separation of a 14-component PS mixture on two 4.6×150 mm columns packed with $1.7 \mu\text{m}$ particles with 200 and $450 \text{ \AA}$ mean pore size. This 4 min separation has more resolution and is about ten times faster than the conventional SEC approach depicted for a similar 12-component mixture in Fig. 6B.

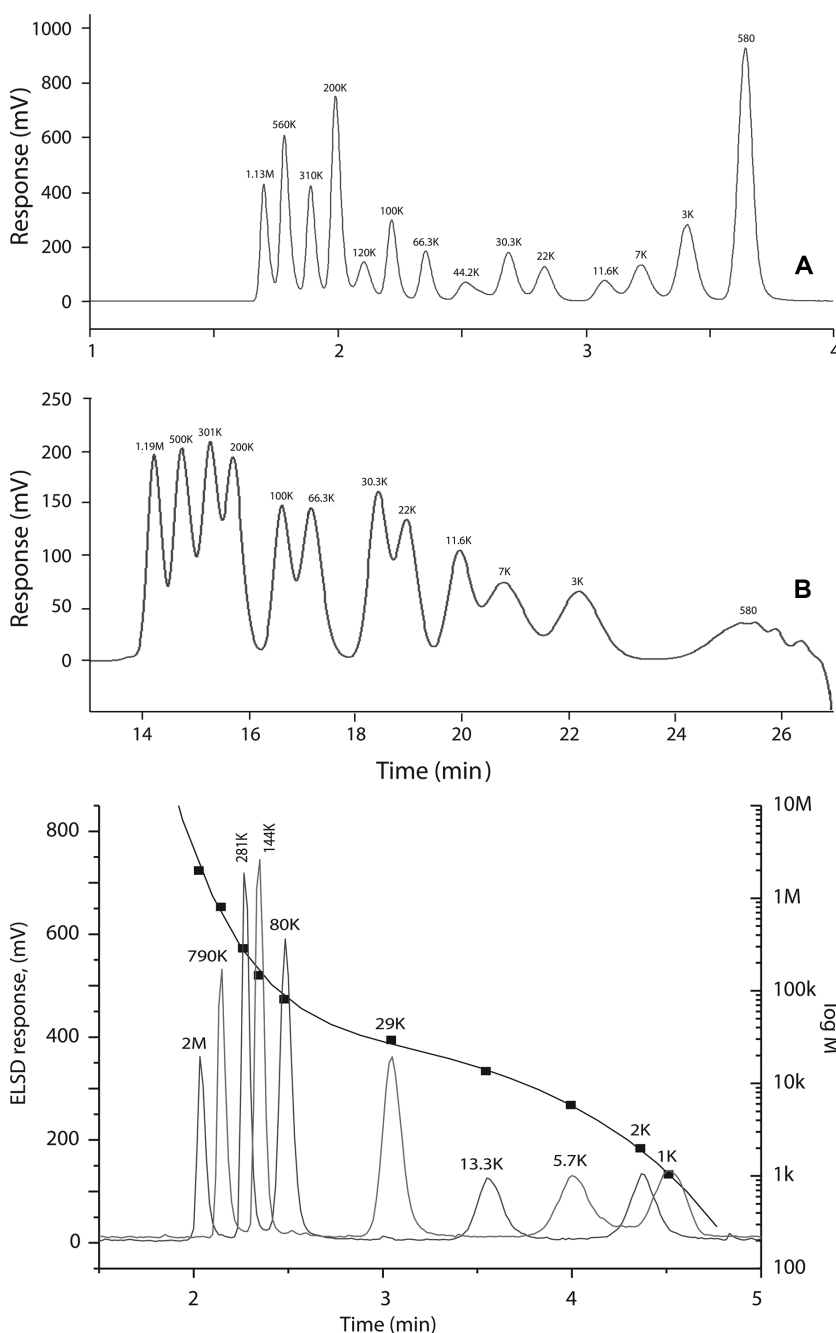


Figure 6. Chromatograms of (A) fourteen-component mixture of PS standards obtained on two 4.6×150 mm columns connected in series. First column, $1.7 \mu\text{m}$ BEH diol 200 $\text{\AA}$; second column, $1.7 \mu\text{m}$ BEH diol 450 $\text{\AA}$. Mobile phase, THF; flow rate, 1 mL/min; detection, ELS, (B) twelve-component PS standards obtained on three PL gel SEC columns (7.5×300 mm each) packed with $5 \mu\text{m}$ PS-divinylbenzene particles with pore sizes labeled as 10E2, 10E3, and 10E4 $\text{\AA}$. Mobile phase, THF; flow rate, 1 mL/min; detection, RI.

Figure 7. Chromatograms of PMMA standards and corresponding calibration curve (fifth-order fit). Mobile phase, THF; flow rate, 0.4 mL/min; column, 4.6×150 mm, packed with $1.7 \mu\text{m}$ diol-bonded particles with a mean pore size of 200 $\text{\AA}$; detection, ELS.

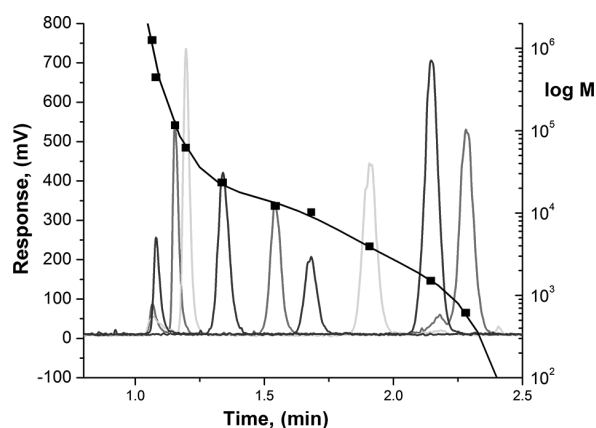


Figure 8. Chromatograms of PEO standards and corresponding calibration curve (fifth-order fit). Mobile phase, 20 mM NH_4Ac in MeOH; flow rate, 1 mL/min; column, same as in Fig. 7; detection, ELS.

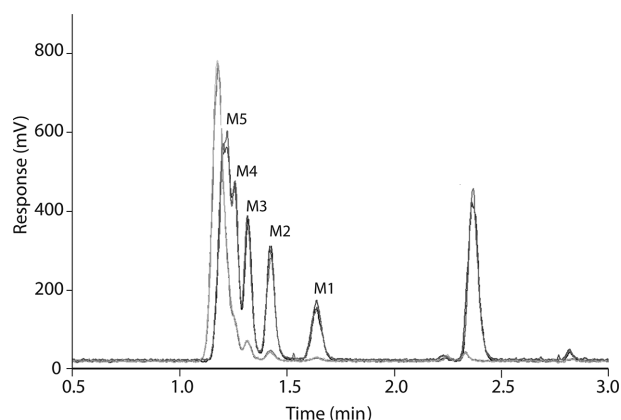


Figure 9. Chromatograms of commercial "polymer C" and "polymer D." Mobile phase, 20 mM NH_4Ac in MeOH; flow rate, 1 mL/min; column, same as in Fig. 7; detection, ELS.

reproducibility of the UHP SEC system. Average M_n and M_w data of polymers C and D were determined by comparing each SEC chromatogram to the PEO calibration curve (fifth-order fit) and determined M values of analyzed samples are summarized in Table 2. M_n and M_w data obtained by conventional SEC collected over about a one year period are also shown for comparison. Good agreement of M data obtained by conventional and UHP modes of SEC has been demonstrated. In UHP SEC mode the %RSD ($n = 4$) is 0.9–1.0% for both M_n and M_w for the commercial polymer samples. This appears to be similar or slightly better than the precision of conventional SEC (typically 3–10%), but further work is required to confirm this observation.

3.5 Limitation of UHP SEC

The separation of synthetic polymers with average molecular weights of several million daltons has always been a challenging task. SEC separations of polymers with high M_w are performed on columns packed with large particles (typically

Table 2. M data of selected commercial polymer samples

Sample id	Average M data determined by					
	Conventional SEC ^{a)}			UHP SEC ^{b)}		
	M_w	M_n	M_w/M_n	M_w	M_n	M_w/M_n
Polymer C	33 000	26 000	1.3	36 000	26 000	1.4
Polymer D	79 000	55 000	1.4	74 000	55 000	1.3

Average of four injections. The %RSD ($n = 4$) for M_w and M_n for both samples by UHP SEC is 0.9–1.0%.

a) In N,N -dimethylacetamide using evaporative light scattering detection. Average of more than 100 injections between July 2008 and July 2009.

b) In 20 mM NH_4Ac in MeOH using evaporative light scattering detection.

20 μm) that are operated at very low flow rates (typically 0.1–0.2 mL/min) to minimize shear stress on the molecules while traveling through the column bed [23].

In order to determine the impact of sub 2 μm particles on separations of high molecular weight polymers, we evaluated separations performed at high linear velocities using high M_w PS on our prototype 1.7 μm particle-packed columns. An example is depicted in Fig. 10A. It shows that a PS standard with M_w of about 4 000 000 Da elutes as a bimodal peak: a minor peak with an M_p of about 2 700 000 Da and a major peak of about 63 000 Da. This suggests that the high shear either deformed the polymer or degraded it into smaller fragments, which elute at longer retention times. To shed more light on this phenomenon, a 4 000 000 Da PS standard that elutes as a bimodal peak was fractionated into two fractions, and then reinjected into a conventional SEC system. The resulting chromatograms (Fig. 10B) show that the 4 000 000 Da standard and its two fractions have similar retention times, eluting at a retention time comparable to the 4 000 000 Da standard. These results suggest that bimodal elution profile of this high M_w standard is consistent with what one would expect for slalom chromatography [24] and not because of mechanical shearing of the polymer chain [25]. This positive result means that this effect can likely be minimized or even eliminated by using lower linear velocities and columns with pores large enough to accommodate high M polymer chains. However, in general, the upper molecular weight limit for other polymers may be different and dependent on their chemistry. Until this limitation is more fully understood, analysis of high-molecular-weight macromolecules on this system should be checked for shear degradation and the onset of slalom chromatography by running appropriate standards or by comparing the results to those obtained on a conventional SEC system.

Another obstacle to the facile acceptance of UHP SEC is the higher than ideal dispersion of commercial refractive index (RI) detectors. The large volume of traditional RI detector flow cells adds substantial dispersion to the chromatographic peaks. A substantial reduction in RI flow cell volume

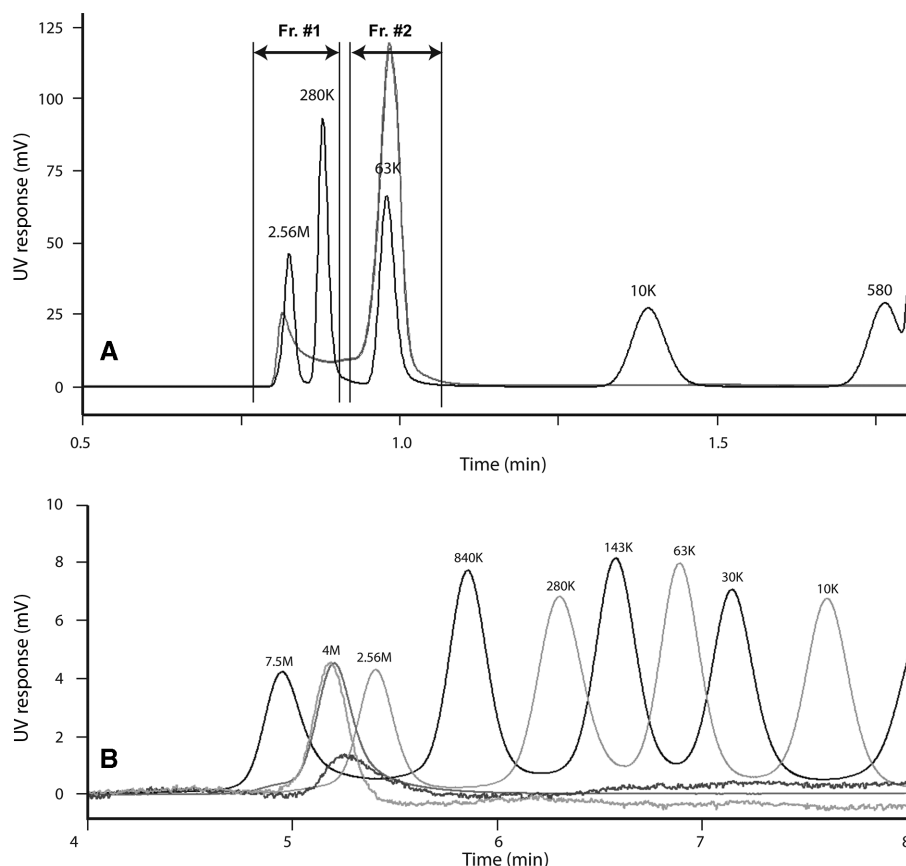


Figure 10. (A) Chromatograms of PS standard mix (black) and individual PS standard with $M_p \sim 4$ M. Column, 4.6×150 mm, $1.7 \mu\text{m}$ with $200 \text{ \AA}$ BEH diol particles; mobile phase, THF; flow rate, 1 mL/min; detection, ELS. The 4 M standard is seen to elute as bimodal peak. The eluent was collected in two fractions and reinjected into (B) two 7.5×300 mm PL Mixed B columns (Agilent, Santa Clara, CA, USA) connected in series, packed with $10 \mu\text{m}$ PS-DVB particles. In addition to Fractions #1 and #2, PS standards were also run; detection, RI. In all cases, mobile phase was THF and flow rate was 1.0 mL/min.

without deleteriously affecting sensitivity would enable UHP SEC separation and quantitation for a wide spectrum of polymers that do not contain UV chromophores. After the limitations of the method have been addressed, we anticipate this technique will be widely applied for typical 1D molecular weight measurements, as well as the second dimension in 2D separations, and in other hyphenated analyses.

4 Concluding remarks

It has been demonstrated that SEC in UHP mode is a feasible approach for the molecular weight characterization of synthetic polymers in organic solvents. Using the Waters ACQUITY® UPLC® H-Class system and BEH SEC columns packed with $1.7\text{--}2.5 \mu\text{m}$ particles and varying pore sizes ($45\text{--}900 \text{ \AA}$), UHP SEC separations of mixtures of PS, PMMA, and PEO standards are achieved within 2–4 min. This speed is about ten times faster than conventional SEC separations. Using corresponding calibration curves, average M data of several polymer samples have been evaluated. Molecular weight values obtained from the UHP SEC system are in good agreement with results obtained using the conventional SEC approach.

The authors have declared no conflict of interest.

5 List of symbols

a, b, c	constants in van Deemter equation
$\mathcal{D}$	dispersity M_w/M_n
D_m	diffusion coefficient
d_p	particle size
H	plate height
K_D	partition coefficient for SEC
M, b	slope and intercept of SEC calibration curve in linear region
M	molecular weight
M_n	number-averaged molecular weight
M_w	weight-averaged molecular weight
u	linear velocity
V_i	intraparticle volume
V_0	interstitial volume
V_R	retention volume

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