Rapid Determination of Molecular Parameters of Synthetic Polymers by Precipitation/Redissolution High-Performance Liquid Chromatography Using "Molded" Monolithic Column

MIROSLAV JANČO,[1] DAVID SÝKORA,[1] FRANTISEK SVEC,[1] JEAN M. J. FRÉCHET,[1] JOHANNES SCHWEER,[2] REIMER HOLM[2]

[1] Department of Chemistry, University of California, Berkeley, California 94720

[2] Bayer AG, Central Research and Development, D-51368 Leverkusen, Germany

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ABSTRACT: Rapid high-performance liquid chromatography (HPLC) of polystyrenes, poly(methyl methacrylates), poly(vinyl acetates), and polybutadienes using a monolithic 50 × 4.6 mm i.d. poly(styrene-*co*-divinylbenzene) column have been carried out. The separation process involves precipitation of the macromolecules on the macroporous monolithic column followed by progressive elution utilizing a gradient of the mobile phase. Depending on the character of the separated polymer, solvent gradients were composed of a poor solvent such as water, methanol, or hexane and increasing amounts of a good solvent such as THF or dichloromethane. Monolithic columns are ideally suited for this technique because convection through the large pores of the monolith enhances the mass transport of large polymer molecules and accelerates the separation process. Separation conditions including the selection of a specific pair of solvent and precipitant, flow rate, and gradient steepness were optimized for the rapid HPLC separations of various polymers that differed broadly in their molecular weights. Excellent separations were obtained demonstrating that the precipitation-redissolution technique is a suitable alternative to size-exclusion chromatography (SEC). The molecular weight parameters calculated from the HPLC data match well those obtained by SEC. However, compared to SEC, the determination of molecular parameters using gradient elution could be achieved at comparable flow rates in a much shorter period of time, typically in about 1 min. © 2000 John Wiley & Sons, Inc. J Polym Sci A: Polym Chem 38: 2767–2778, 2000

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INTRODUCTION

Size exclusion chromatography[1] (SEC) and high-performance liquid chromatography[2] (HPLC) are currently the dominant techniques used for the separation and characterization of polymers, although somewhat analogous alternative methods such as electrophoresis,[3] field-flow fractionation,[4] and hydrodynamic chromatography[5] relying on different separation mechanisms have also been developed.

SEC is preferred for the separation and characterization of polymers according to their molec-

Correspondence to: J. M. J. Fréchet

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ular weight characteristics as a result of its universality, good accuracy, reliability, reproducibility, and low sample consumption. The separation mechanism in SEC utilizes differences in entropy and the separation of macromolecules is achieved according to their hydrodynamic volumes in a specific solvent. Since Einstein's viscosity law defines the hydrodynamic volume as a product of molecular weight and intrinsic viscosity,[6] SEC affords molecular weight parameters of analyzed polymers. However, SEC is not able to efficiently separate macromolecules of the same size that may differ in chemical composition, physical structure, or even molecular weight. The resolution in the SEC separations is determined by pore volume of the column packing. Because of the simplicity of the SEC technique, the number of adjustable parameters enabling its tuning is limited.

High-performance liquid chromatography (HPLC) can also be used for the selective separation and characterization of polymers. The separation of polymers in HPLC is based on differences in enthalpy resulting from their interactions with the stationary phase such as adsorption and partition,[7–9] or from differences in their solubility typical of the precipitation/redissolution mode.[10,11] As a rule, the retention factor k in a fixed solvent increases exponentially with increasing molecular weight of polymers and the retention times of large molecules might be too long for practical use. Therefore, isocratic elution with a single solvent is limited to the separations of oligomers,[8,12,13] although the isocratic separation of high molecular weight polymers has also been attempted.[14–16] In contrast, elution using a mobile phase with an increasing solvency[17–21] or a gradient of temperature[22–25] enables separations of both oligomers and polymers in a much shorter period of time.

In addition to the determination of molecular weight, HPLC can also separate polymers according to their chemical composition.[26] Because of the specific requirements such as the need to optimize separation conditions for each individual polymer, re-equilibration between consecutive runs, and method validation for each chromatographic system, HPLC currently only complements the more popular SEC. In contrast to SEC, HPLC commonly offers much higher resolution and a larger number of variables that control the chromatographic process such as flow rate, mobile phase composition, gradient profile, and temperature, can be used to fine-tune the separation.

Although known since the 1950s, the concept of polymer precipitation followed by successive redissolution in a gradient of the mobile phase has only recently been exploited for the high-performance chromatography of polymers.[11,26,27] The procedure begins by injection of a polymer solution into a stream of the mobile phase in which the polymer is not soluble. Therefore, all of the macromolecules precipitate at the top of the column and form a separate gel phase that adheres to the surface of the packing and does not move. As the solvency of the mobile phase is gradually increased, macromolecules with the lowest molecular weight start to dissolve and move within the stream of the mobile phase. A continuing increase in the percentage of good solvent in the mobile phase enables dissolution of polymers with increasing molecular weights. As a result, the polymer molecules are eluted in an order that is opposite to that of typical SEC. Smaller molecules elute first followed by the larger ones. Under ideal conditions, the column packing should serve only as a support for the precipitated phase, and therefore, the separation should not depend on the chemistry of the separation medium. However, this is not always the case and interactions between analyte and column packing may play a significant role even in the precipitation/redissolution separation process.[11, 26–28]

Typical SEC separation run times are often in the range of several tens of minutes since the flow rate is low (typically 1–2 mL/min) to achieve the highest column efficiency and a series of rather large columns (often 2–4 columns, each 300 $\times$ 8 mm i.d.) with large void volumes are used to achieve the desired resolution. Long run times are ill-suited for applications such as the high throughput screening of large numbers of samples produced by combinatorial techniques or in-line control of polymerization processes in which the speed of the analysis is the most important issue. Despite one recently reported success,[29] substantial acceleration of true SEC separations remains difficult to achieve.

In contrast to SEC separations, which typically run at an optimal flow rate that should not be changed, several variables of the gradient elution process can be adjusted without decreasing the resolution. For example, the speed of the separation may be increased considerably using high flow rates and steep gradients. However, high flow rates are not possible for columns packed with small microparticles as they often exhibit prohibitively high flow resistance. The monolithic

columns that we introduced in the early 1990s[30,31] tolerate very high flow velocities since their resistance to flow is very low. We have already demonstrated that the separation media consisting of "molded" porous monoliths can be used for the separations of styrene oligomers and polymers according to their molecular weight.[32,33] Monolithic columns are well suited for this separation technique because convection through the large pores of the monolith enhances the mass transport of large analyte molecules and accelerates the separation process. The introduction of monolithic columns is instrumental for making precipitation/redissolution chromatography a viable technique for the characterization of polymers. The extremely good permeability of monolithic columns enables separations of polymers to be achieved at remarkable speeds. For example, three polystyrene standards were baseline separated on a 50 × 8 mm i.d. monolithic column in only 16 s using a flow rate of 20 mL/min.[32]

This report demonstrates the applicability of HPLC on monolithic columns for the rapid determination of molecular parameters of synthetic polymers using the precipitation/redissolution technique at much lower flow rates than has been previously described. This is achieved by using columns with a smaller diameter and optimized gradients of the mobile phase. In addition to polystyrenes, we also demonstrate separations of poly(methyl methacrylates), poly(vinyl acetates), and polybutadienes and compare the results of these rapid separations with data obtained using SEC.

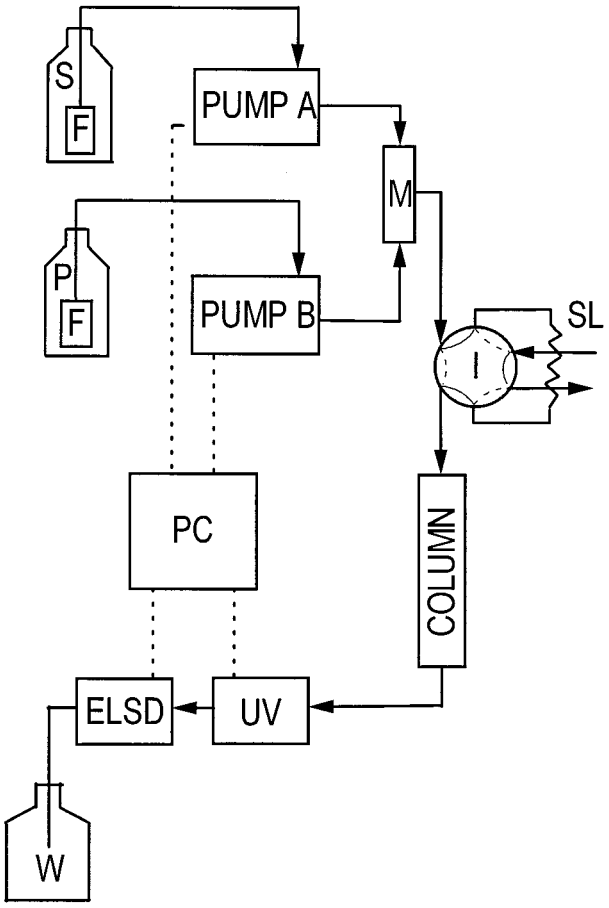

Figure 1. Schematic representation of the HPLC system. S, P: solvent and precipitant reservoirs; M: 250 μL binary mixer; I: manual injector; SL: 10 μL sample loop; F: solvent filters; W: waste reservoir.

EXPERIMENTAL

Materials

Low polydispersity polystyrene, poly(methyl methacrylate) (both from Polymer Laboratories, Church Stretton, UK), polybutadiene (Polymer Standard Services, Mainz, Germany), and "secondary" poly(vinyl acetate) (Aldrich, Milwaukee, WI) molecular weight standards were used for the determination of optimal separation conditions and method calibration. Commercial polystyrenes were obtained from Rhodia (Aubervillier's Research Center, France) and Bayer (Leverkusen, Germany) or purchased from Kodak (Rochester, NY), Scientific Polymer Products (sp^2, Ontario, NY), and Aldrich. Isotactic poly(methyl methacrylates) prepared by anionic polymerization at low temperature were obtained from Osaka University (Japan). Tetrahydrofuran (THF), dichloromethane, methanol, and *n*-hexane (all HPLC grade, Fischer, CA) were used as delivered.

HPLC

Precipitation redissolution chromatography was carried out using a HPLC system shown schematically in Figure 1 consisting of two 515 pumps (Waters, Milford, MA) connected through a 250 μL binary T mixer assembly (Analytical Scientific Instruments, Richmond, CA), an injection valve (Rheodyne, Cotati, CA) with a 10 μL sample loop, and two detectors, a 486 UV detector (Waters) and an evaporative light scattering detector (ELSD) (950 EMD, Polymer Laboratories, Church Stretton, UK) connected in series. The injector and column inlet were connected with a short

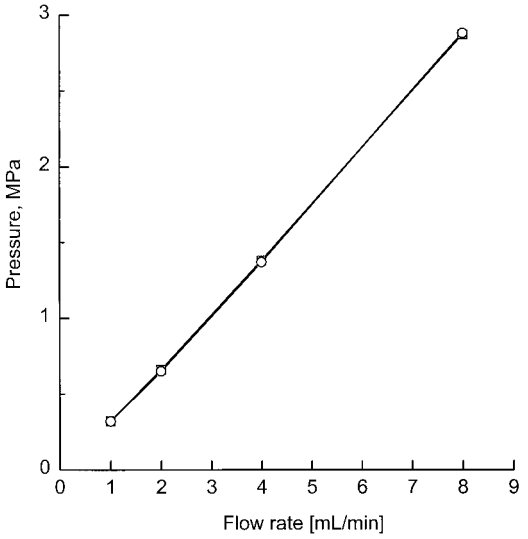

Figure 2. Back pressure in the monolithic 50 × 4.6 mm i.d. poly(styrene-*co*-divinylbenzene) column as a function of both increasing (□) and decreasing (○) THF flow rate.

stainless steel capillary (50 mm) to avoid precipitation of the polymer within the inlet line. System control, data acquisition, and data processing were performed using the Millennium 2010 software (Waters). Alternatively for some separations, a model 2690 XE Alliance separation module (Waters) controlled by Millennium 2.15 software equipped with a Rheodyne injector for manual injection was used.

Monolithic Column

All precipitation/redissolution separations were performed using a 50 × 4.6 mm i.d. macroporous poly(styrene-*co*-divinylbenzene) monolithic column that was prepared using the technique published elsewhere.[34] These columns are now available from ISCO, Inc. (Lincoln, NE). Linearity of the back pressure versus flow rate plots shown for both increasing and decreasing flow rates in Figure 2 confirm both the low flow resistance and the lack of compressibility of this column.

SEC

SEC measurements were carried out in THF at a flow rate of 1 mL/min using a model 2690 XE Alliance separation module (Waters) equipped with a column set comprising two 300 × 7.5 mm i.d. 10^3 Å and 10^5 Å PL gel columns (Polymer Laboratories). Typically, 20 μL polystyrene or 50 μL poly(methyl methacrylate) solutions (concentration 1–2 mg/mL) were injected using the built-in autoinjector. The signals obtained from both the 486 UV and the 950 EMD detectors were used for the calculation of molecular weights.

Cloud Point Determination

Cloud points were determined visually by titration of a 5 mg/mL polymer solution in a good solvent with a specific precipitant at room temperature.

RESULTS AND DISCUSSION

Molecular weight determination by precipitation/redissolution HPLC is a relative method and, therefore, must be calibrated. Typically, the calibration is performed by determining retention times of polymer standards with narrow molecular weight distribution. The nominal molecular weight is then plotted against the retention time to obtain the desired calibration curve. To satisfy a special requirement of the Waters software, the calibration curve should span as broad a range of molecular weights as possible to ensure that the molecular parameters of the future "unknown" samples will fall within this range. Since no "universal" calibration currently exists for the gradient HPLC technique, standards of the same chemical composition as the analyzed samples must be used for the calibration. This requirement restricts the precipitation/redissolution analysis to polymers for which standards with well-defined molecular weights are available.

Polystyrene

Linear Gradient

Based on our previous research,[32,33] a gradient of THF (solvent) in methanol (precipitant) was used for the separation of polystyrenes according to their molecular weight in a solvent gradient. The separations were first carried out with a simple 5 min linear gradient from 0 to 60% THF in methanol at a flow rate of 1 mL/min. Figure 3 shows that the programmed gradient corresponds well to that monitored by the UV detector. The shift in the starting point of the monitored gradient represents the hold-up volume of the chromatographic system. This gradient enables the separation of polystyrene standards with molecular

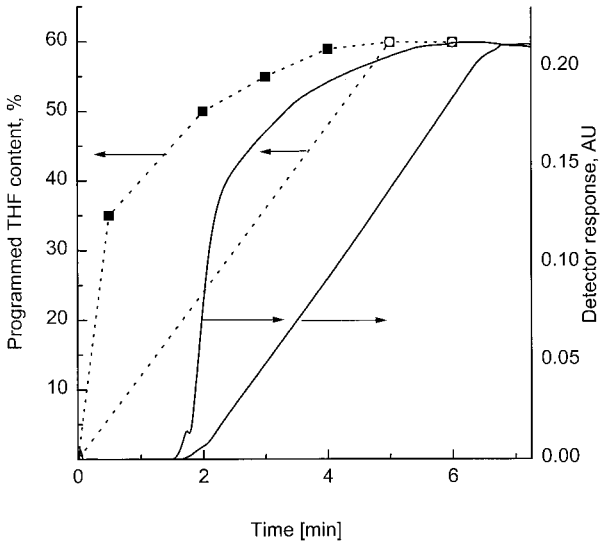

Figure 3. Plots of the programmed linear (○) and the stepwise gradients (■) of 0–60% THF in methanol used for the separation of polystyrenes (dotted lines) together with the change in composition of the mobile phase as monitored by the UV detector (solid lines).

weights ranging from 3000 to 980,000 as shown in Figure 4. The calibration curve, also shown in Figure 4, has an exponential shape. This shape is not favorable since the resolution for polymer molecules of higher molecular weight is lower than that for molecules with lower molecular weight. This "nonlinearity" also affects the peak shape of samples of higher molecular weight. The peak shape is not Gaussian and appears to be somewhat compressed. Using standard SEC software, this renders the direct and simple calculation of molecular parameters less reliable for higher molecular weight polymers. The performance of the separations was tested using binary mixtures of two different narrow molecular weight polystyrene standards. These binary mixtures of standards were used to validate the absence of mutual effects of polymer molecules of different molecular weights on both the precipitation and the redissolution process. Table I compares molecular weight characteristics for the components of binary model mixtures calculated from elution profiles obtained by both the HPLC techniques using a linear gradient and the conventional SEC method. Molecular weight data obtained from gradient elution of standards with lower MW are slightly higher than those calculated from SEC. In contrast, the molecular weight parameters of high molecular weight standards obtained from gradient elution are generally much smaller because of the peak tail compression effect resulting from the nonlinearity of the calibration curve.

Nonlinear Gradient

In order to avoid the undesired compression of the peaks, we designed a nonlinear gradient with a profile that compensates for the decreasing resolution in the high molecular weight region and affords a linear calibration curve. Cloud point curves shown in Figure 5 were obtained by titration of a THF solution of polystyrene standards with methanol. These profiles are well-suited guide to the design of the gradient shape. The optimized gradient profile shown in Figure 3 then follows the shape of the percentage of THF versus molecular weight curve. It is composed of five linear ramps steeper at the beginning and shallower at the end. An excellent separation of a mixture of nine polystyrene standards shown in Figure 6(a) is achieved using this "tailored" nonlinear gradient within 6.5 min at a flow rate of 1 mL/min. In contrast, the SEC separation of this mixture using a tandem of two columns at the same flow rate shown in Figure 6(b) requires 18

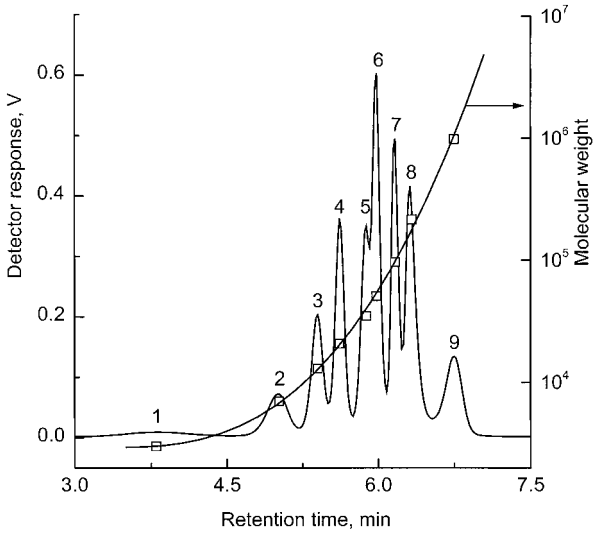

Figure 4. Separation of a mixture of nine polystyrene standards using a monolithic column and the corresponding calibration curve obtained from the elution data. Separation conditions: linear gradient, 0–60% THF in methanol in 5 min; flow rate, 1 mL/min; sample volume, 10 μL; overall sample concentration, 18 mg/mL (2 mg/mL of each standard) in THF; ELSD detection. Molecular weights of polystyrene standards: 3000 (1), 7000 (2), 12,900 (3), 20,650 (4), 34,800 (5), 50,400 (6), 96,000 (7), 214,500 (8), and 980,000 (9).

Table I. Molecular Parameters of Individual Polystyrene Standards with Narrow Molecular Weight Distribution as Calculated from the Separations of Their Binary Mixtures Determined by HPLC and SEC Using an Evaporative Light Scattering Detector

Binary Polymer Mixture[a]	$M_w \times 10^{-3}$			$M_n \times 10^{-3}$			M_w/M_n		
	HPLC[b]			HPLC[b]			HPLC[b]		
	Linear	Stepwise	SEC	Linear	Stepwise	SEC	Linear	Stepwise	SEC
18.1k	18.9	17.7	17.4	18.5	17.6	17.2	1.01	1.01	1.01
150k	139.8	153.4	148.5	137.8	152.1	146.1	1.01	1.01	1.02
30k	32.6	28.9	30.1	32.3	28.7	29.9	1.01	1.01	1.01
200k	168.9	211.4	211.6	166.5	209.4	209.9	1.01	1.01	1.01

[a] Values provided by manufacturer.
[b] HPLC separations were carried out on a monolithic column using the linear or the five-step gradient shown in Figure 3. For other separation conditions see Experimental.

min. The HPLC separation of polystyrene standards affords a trace with the expected order of elution: Lower molecular weight polymers elute prior to higher ones. Larger macromolecules with a higher number of hydrophobic repeat units are retained longer and their elution requires a higher percentage of good solvent in the mobile phase. This sequence is quite unlike size-exclusion chromatography for which large molecules elute first. Because of the opposite order of elution, the chromatograms in Figure 6 are nearly mirror images.

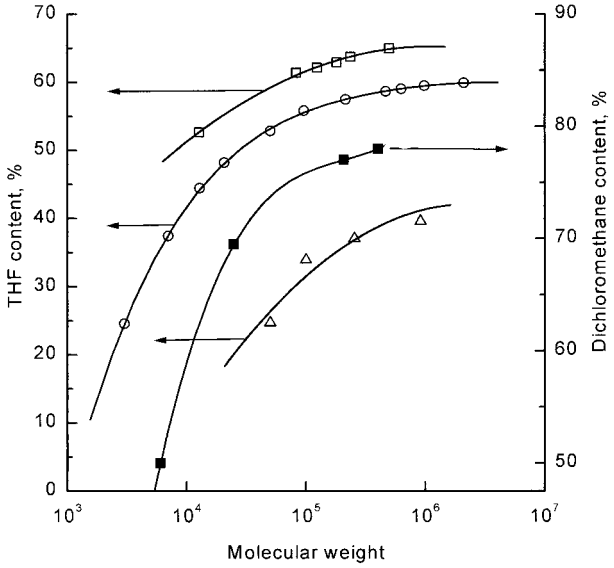

Figure 5. Effect of molecular weight on the solubility of poly(vinyl acetate) in THF/*n*-hexane (□), polystyrene in THF/methanol (○), poly(methyl methacrylate) in THF/methanol (△), and polybutadiene in dichloromethane/methanol (■) mixtures. Cloud points were determined visually by titration of 5 mg/mL solutions of polymer in good solvent with a precipitant at ambient temperature.

Figure 6 also shows calibration curves (molecular weight vs. retention time) for both methods. The HPLC calibration is linear over the entire range of measured molecular weights. The accuracy of this HPLC method featuring the nonlinear gradient elution for molecular weight determinations was examined again using the binary mixtures of two polystyrene standards. Table I demonstrates that this time the molecular parameters calculated from both HPLC and SEC traces are very close and match well the nominal molecular weights provided by manufacturer.

Although the results obtained for narrow molecular weight standards were adequate, the real test of suitability of this method is the separation of common polymers with a broader molecular weight distribution. We separated several polystyrenes from different sources using both precipitation/redissolution and SEC techniques and calculated their molecular weight parameters. Table II shows that despite the differences in separation mechanisms, an excellent match is obtained. Lower polydispersity values calculated from HPLC data may result from peak compression in the HPLC mode and/or band broadening in the SEC mode. Here again, the HPLC measurement is completed in about 6 min with a provision for a further acceleration (*vide infra*), while the complete SEC trace is eluted only after 20 min.

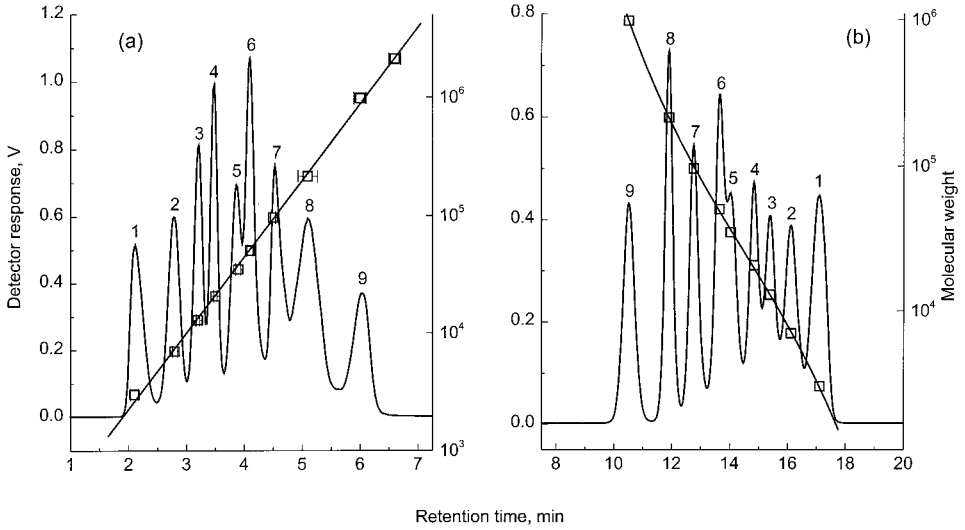

Figure 6. Separation of a mixture of nine polystyrene standards using (a) HPLC in a monolithic 50 × 4.6 mm i.d. poly(styrene-*co*-divinylbenzene) column or (b) SEC in two PL gel column (300 × 7.5 mm i.d.) together with the corresponding calibration curves obtained from the elution data. Separation conditions: flow rate, 1 mL/min; overall sample concentration, 18 mg/mL (2 mg/mL of each standard) in THF; ELSD detection. HPLC eluent: nonlinear gradient consisting of 0–35% THF in methanol in 0.5 min, 35–50% in 1.5 min, 50–55% in 1 min, 55–59% in 1 min, and 59–60% in 1 min. SEC eluent: THF. Molecular weights of polystyrene standards: 3000 (1), 7000 (2), 12,900 (3), 20,650 (4), 34,800 (5), 50,400 (6), 96,000 (7), 214,500 (8), and 980,000 (9).

Acceleration of the Separation

Gradient elution is controlled by a number of variables that can be varied to accelerate the separation. According to eq 1[35] the average retention factor in gradient elution, $\bar{k}$, depends on the gradient time, t_G, the flow rate, F, the difference in composition of the mobile phase D_f, the column dead volume V_m, and a constant, S, which is calculated for each solute from the retention data in isocratic systems and characterizes the strength of the interaction between the solute and the stationary phase,

$$\bar{k} = t_G \cdot F/D_f \cdot V_m \cdot S \qquad (1)$$

For a constant range of the mobile phase composition and for a specific column and solute, the denominator of eq 1 remains constant and the average retention factor, $\bar{k}$, only depends on the gradient time t_G and the flow rate F that can be

Table II. Molecular Parameters of Polystyrenes as Determined by SEC and HPLC

	$M_w \times 10^{-3}$		$M_n \times 10^{-3}$		M_w/M_n	
Origin	HPLC[a]	SEC	HPLC[a]	SEC	HPLC[a]	SEC
Rhodia	73.4	72.5	67.2	67.7	1.09	1.08
Sp[2]	211.3	208.1	159.8	147.3	1.32	1.41
Aldrich 1	266.1	285.1	202.3	202.6	1.32	1.41
Bayer 1	290.2	297.5	213.4	206.5	1.36	1.44
Kodak	293.1	311.8	227.9	221.2	1.29	1.41
Aldrich 2	294.0	330.5	204.5	211.4	1.44	1.56
Bayer 2	302.3	319.9	225.7	223.2	1.34	1.43

[a] HPLC separations were carried out on a monolithic column using the five-step gradient shown in Figure 3 and an evaporative light scattering detector. For other separation conditions see Experimental.

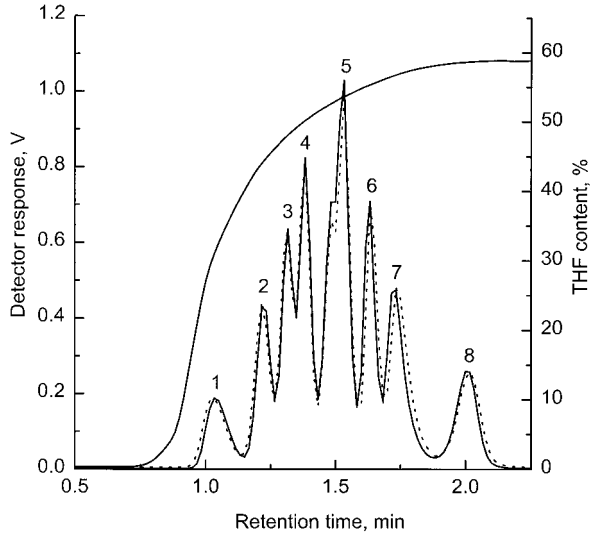

Figure 7. Rapid separation of a mixture of eight polystyrene standards using a monolithic poly(styrene-*co*-divinylbenzene) column and the corresponding gradient profile monitored by the UV detector. Separation conditions: 1.25 min gradient of THF in methanol consisting of 0–35% THF in methanol in 0.12 min, 35–50% in 0.38 min, 50–55% in 0.25 min, 55–59% in 0.25 min, and 59–60% in 0.25 min; overall sample concentration, 16 mg/mL (2 mg/mL of each standard) in THF; ELSD detection. Molecular weights of polystyrene standards: 3000 (1), 7000 (2), 12,900 (3), 20,650 (4), 50,400 (5), 96,000 (6), 214,500 (7), and 980,000 (8).

used to control the separation. For example, Figure 7 demonstrates the considerable acceleration in the separation of polystyrene standards achieved by simply doubling the flow rate to 2 mL/min and by decreasing the gradient time by a factor of 4 to 1.25 min. The complete separation of this mixture is achieved within 1 min.

Sample Load

Numerous reports related to the gradient elution of macromolecules indicate a large effect of sample load on peak shapes and retention times.[7,11,18,36,37] Our early work with styrene oligomers demonstrated that this effect was much less evident for separations involving monolithic columns.[33] Figure 8 shows the separations of five polystyrene standards injected in solutions with different overall concentrations ranging from 2.5 to 50 mg/mL. The retention times for all of the peaks remain almost constant over the entire load range. For example, no change was observed for the first two peaks while a difference of only 13 s in the retention times (less than 4%) was monitored for the standard with molecular weight 629,000. Although this difference is very small,

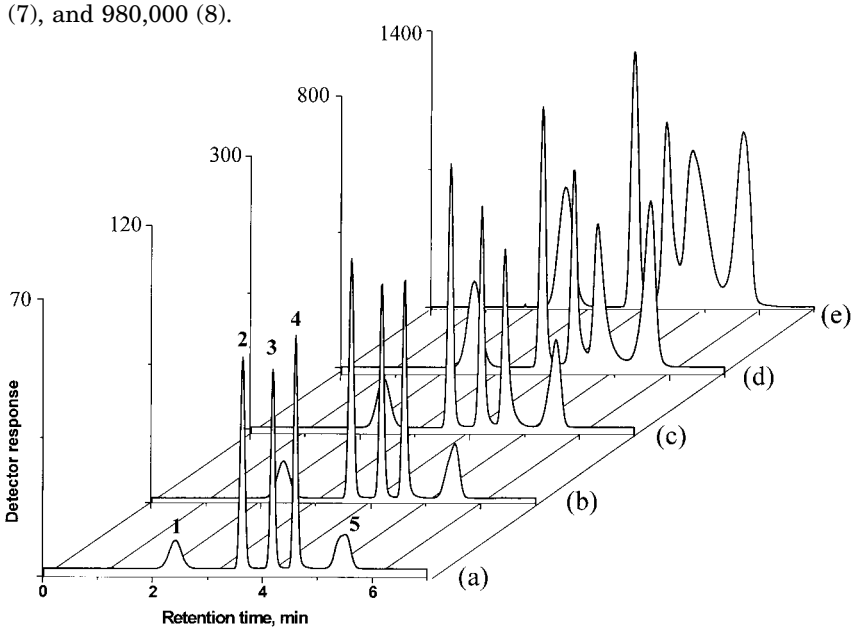

Figure 8. Effect of sample concentration on retention times of polystyrene standards. Column: 50 × 4.6 mm i.d. poly(styrene-*co*-divinylbenzene) monolith; 5 min nonlinear gradient consisting of 0–35% THF in methanol in 0.5 min, 35–50% in 1.5 min, 50–55% in 1 min, 55–59% in 1 min, and 59–60% in 1 min; flow rate, 1 mL/min; sample volume, 10 μL; overall concentration: 2.5 (a), 5.0 (b), 10.0 (c), 25.0 (d), and 50 mg/mL (e) in THF; ELSD detection. Molecular weights of polystyrene standards: 4950 (1), 30,300 (2), 66,000 (3), 135,000 (4), and 629,000 (5).

the concentrations of solutions used for calibration and those used for the determination of molecular parameters of an "unknown" sample should be kept close to warrant that accurate results are obtained. Despite the slight deterioration in resolution observed for the highest loading, the separations shown in Figure 8 also demonstrate the broad dynamic range of the monolithic column and suggest that even small monolithic columns may be useful for the preparative separation of polymers.

Higher column loading can also be achieved by injecting larger volumes of samples. However, comparative experiments document that this approach leads to a larger shift in retention times for high molecular weight polymers. In addition, larger injection volumes introduce larger volumes of the good solvent into the system thereby triggering the undesired preelution.[18,26]

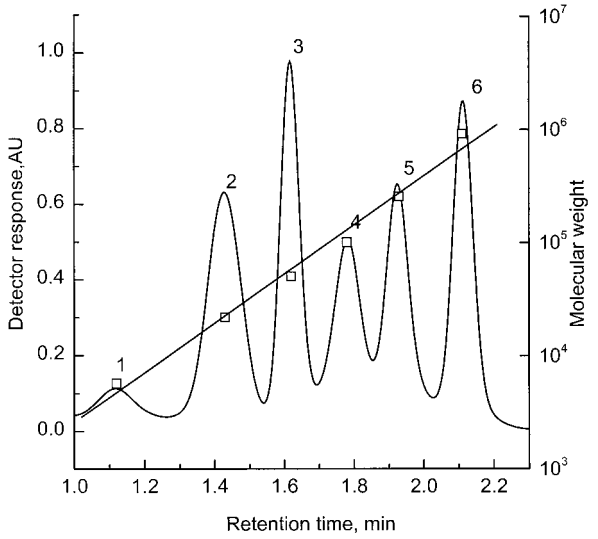

Figure 9. Rapid separation of a mixture of six poly(methyl methacrylate) standards using a monolithic poly(styrene-*co*-divinylbenzene) column and the corresponding calibration curve obtained from the elution data. Separation conditions: 2 min gradient of THF in methanol consisting of 0–35% THF in methanol in 1 min and 35–50% in 1 min; flow rate, 1 mL/min; sample volume, 10 μL; overall sample concentration: 30 mg/mL in THF; UV detection at 220 nm. Molecular weights of polymer standards: 5750 (1), 21,650 (2), 50,000 (3), 100,000 (4), 254,000 (5), and 910,000 (6).

Separations of Other Polymers

Poly(methyl methacrylate)

Methacrylate polymers are more polar than polystyrene and their solubility parameters may also be expected to be different. In order to characterize poly(methacrylates) it is necessary to perform the steps of cloud point titration (Fig. 5), optimization of the gradient shape, and calibration with standards. In this study, a much simpler two-step gradient of THF in methanol was sufficient to "linearize" the calibration curve since no very low molecular weight standards were included. Figure 9 shows the rapid separation of 6 PMMA standards with molecular weights in the range 5750–910,000. This separation is accomplished within 1.2 min at a flow rate of only 1 mL/min. Using the optimized gradient, molecular parameters of two "unknown" isotactic poly(methyl methacrylates) were determined and compared with those obtained from SEC. The results are summarized in Table III. Although the values of molecular weight and polydispersity determined by both HPLC and SEC do not match exactly, they are again very close. However, the HPLC data are obtained in about one tenth the time required to record the complete SEC trace.

Poly(vinyl acetate)

Since a commercial source of narrow poly(vinyl acetate) standards was not readily available, the separations were carried out with broad "secondary" standards available from Aldrich. In contrast to polystyrene and poly(methyl methacrylate), methanol is a solvent for poly(vinyl acetate). Therefore, a series of solvent and precipitant pairs were tested. Molecular weight dependent elution was achieved with THF/water, dichloromethane/*n*-hexane, and THF/*n*-hexane. Optimization of the gradient elution was then carried out with THF and hexane. Figure 10 shows the HPLC traces for broad "secondary" standards and compares them with their SEC counterparts. The HPLC separation appears to be more sensitive to changes in molecular weights than the SEC separation. Unlike the separations of polystyrene and poly(methyl methacrylate), an acceleration of the poly(vinyl acetate) elution cannot be achieved using means such as changes in flow rate and in gradient steepness without a concomitant rapid deterioration of the selectivity.

Polybutadiene

Since THF/methanol did not perform well in the separation of polybutadienes in HPLC mode, dichloromethane was used as the solvent in combination with methanol (precipitant) to achieve the

Table III. Molecular Parameters of Isotactic Poly(methyl methacrylates) Determined by HPLC and SEC

	$M_w \times 10^{-3}$		$M_n \times 10^{-3}$		M_w/M_n	
Polymer	HPLC[a]	SEC	HPLC[a]	SEC	HPLC[a]	SEC
PMMA 1	31.2	33.0	29.1	29.4	1.07	1.12
PMMA 2	128.5	125.7	111.0	95.7	1.16	1.31

[a] HPLC separations were carried out on a monolithic column using a two-step gradient of THF in methanol (0–35% in 1 min + 35–50% in 1 min), flow rate 1 mL/min, UV detection at 220 nm. For other separation conditions see Experimental.

molecular weight-dependent separation. The optimized five-step gradient profile once again affords a linear calibration curve for all five polybutadiene standards available. The separation of these standards is shown in Figure 11. The run time is about 6 min at a flow rate of 1 mL/min.

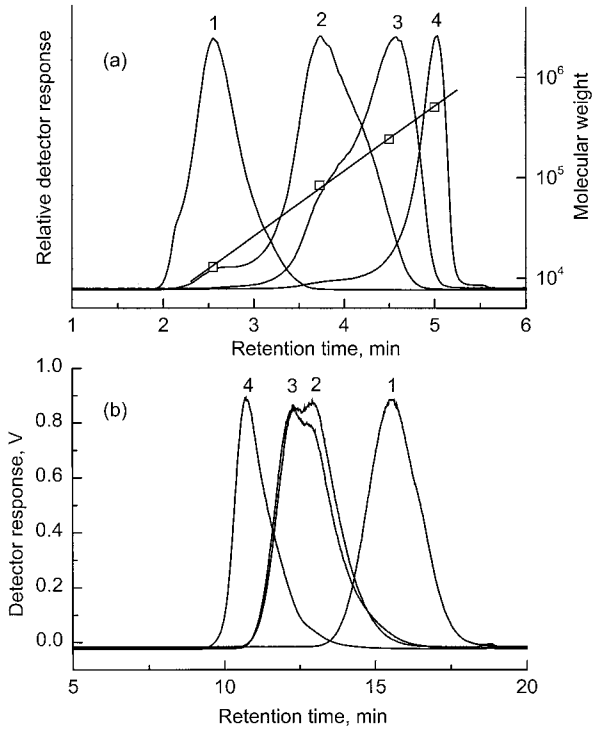

Figure 10. Elution traces of individually injected secondary poly(vinyl acetate) standards using (a) HPLC in a monolithic 50 × 4.6 mm i.d. poly(styrene-*co*-divinylbenzene) column or (b) SEC in two PL gel columns (300 × 7.5 mm i.d.) and the corresponding HPLC calibration curve obtained from the elution data. Separation conditions: 5 min gradient consisting of 0–46% THF in hexane in 1 min and 46–60% in 1.2 min, 60–67% in 1.8 min, and 67–100% in 1 min; flow rate, 1 mL/min; sample volume, 10 μL; sample concentration: 5 mg/mL; ELSD detection. Molecular weights: 12,800 (1), 83,000 (2), 12,900 (3), and 500,000 (4).

However, doubling the flow rate to 2 mL/min and halving the gradient time enables the separation to be achieved within 3 min. A further acceleration is possible by running the gradient within only 1.25 min. Although the resolution of peaks 2 and 3 is slightly lower in this rapid run, the complete separation of the mixture of polybutadiene standards is achieved within 1 min (Fig. 11).

Column Stability

All of the measurements described were carried out using a single 50 × 4.6 mm poly(styrene-*co*-divinylbenzene) monolithic column over a period of about seven months with approximately 3000 injections. In each gradient run one component of the mobile phase was a good swelling agent for the material of the column while the other was a precipitant. Although the high level of crosslinking does not allow extensive swelling of the monolithic material, even small volumetric changes of the matrix constitute a periodic stress for the column. However, this repeated stress had no effect on long-term column performance. During the course of this study the flow rate, one of the most critical variables, was changed quite often, routinely reaching values of up to 8 mL/min. Use of such a high flow rate would not be feasible for a column of this size packed with HPLC grade particles due to the prohibitively high back pressure that would result. Great column stability was also demonstrated by the use of several different solvents such as THF, dichloromethane, methanol, hexane, and water with repeated changes in gradient composition without any adverse effects on the separations. An occasional low flow rate flushing with THF was the only "maintenance" carried out on the column. During the entire period of study, no change in back pressure, flow, and separation characteristics were observed for the monolithic column. Figure 7 shows two HPLC separations of a mixture of eight

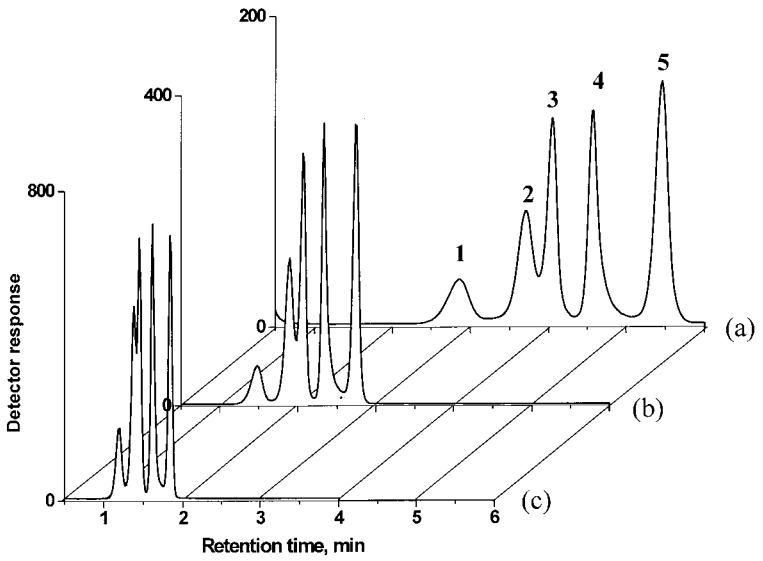

Figure 11. Effect of gradient time and flow rate on the separation of a mixture of five polybutadiene standards using a monolithic poly(styrene-*co*-divinylbenzene) column. Separation conditions: nonlinear gradients of dichloromethane in methanol (a) overall gradient time 5.5 min consisting of 0–75% in 3 min, 75–79% in 0.5 min, 79–81% in 0.5 min, 81–84% in 1 min, and 84–100% in 0.5 min; (b) overall gradient time, 2.75 min consisting of 0–75% in 1.5 min, 75–79% in 0.25 min, 79–81% in 0.25 min, 81–84% in 0.5 min, and 84–100% in 0.25 min; (c) overall gradient time, 1.38 min consisting of 0–75% in 0.75 min, 75–79% in 0.13 min, 79–81% in 0.12 min, 81–84% in 0.25 min, and 84–100% in 0.13 min; flow rate 1 (a) and 2 mL/min (b,c); sample volume, 10 μL; overall sample concentration: 25 mg/mL (each standard 5 mg/mL) in THF; ELSD detection. Molecular weights of polymer standards: 520 (1), 2820 (2), 6000 (3), 24,800 (4), and 215,800 (5).

polystyrene standards recorded more than two months and about 400 injections apart. Even after such a long time the very small difference that can be observed between these two runs lies within the experimental error of chromatographic measurements.

CONCLUSIONS

The simplicity of the preparation, unique flow properties, and enhanced mass transport ability of monolithic columns makes them attractive as an alternative to particulate column packings for the separation of macromolecules. Small monolithic poly(styrene-*co*-divinylbenzene) columns that can be used for the rapid and efficient determination of molecular weight parameters of synthetic polymers in a gradient elution HPLC mode constitute a viable, less expensive, and much faster alternative to the costly sets of sophisticated size-exclusion columns. Although we report the separations of only four different types of polymers, the short molded monoliths are likely to be useful for the rapid separations of many other polymers using optimized gradients of suitable pairs of solvents and nonsolvents. The sample loading affects the separation much less than in the case of packed beds. This is a promising feature for future separations of polymers on the preparative scale.

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