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Molded continuous poly(styrene–*co*-divinylbenzene) rod as a separation medium for the very fast separation of polymers Comparison of the chromatographic properties of the monolithic rod with columns packed with porous and non-porous beads in high-performance liquid chromatography of polystyrenes

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Abstract

Gradient elution separations of polystyrene standards in a monolithic molded 50×8 mm I.D. poly(styrene–*co*-divinylbenzene) rod column and in 50×8 mm I.D. and 30×4.1 mm I.D. columns packed with porous and non-porous poly(styrene–*co*-divinylbenzene) beads has been carried out. All of these separation media differ in shape and porosity. Excellent separations of eight polystyrene standards were achieved with both the molded monolithic rod and porous beads at moderate flow-rates. However, the monolithic medium proved to be superior for high-speed separations using very steep gradients at a flow-rate of 20 ml/min. Three polystyrene standards were separated in the rod column within 4 s. The separation in the column packed with non-porous beads was poor at flow-rates of 2–8 ml/min, while higher flow-rates led to an unacceptably high back pressure.

Keywords: Poly(styrene–*co*-divinylbenzene); Polymers; Polystyrenes; Monolithic column

1. Introduction

Size-exclusion chromatography (SEC) is the dominant technique for the characterization of the molecular size of polymers used routinely in both research and industry [1,2]. Another method that also provides separation of homopolymers according to molecular weight is interactive high-performance liquid chromatography (HPLC). In contrast to the isocratic elution that is characteristic of SEC, HPLC of polymers usually requires a gradient of the mobile phase. This is because the differences in retention for polymers of varying sizes under isocratic conditions are often too large. Several mechanisms for gradient HPLC of polymers have been suggested in the literature [3,4]. However, only two seem to be relevant: (i) conventional retention due to the adsorption that is typical of normal-phase and reversed-phase chromatography and holds for systems that involve low sample loads and separation media with large pores [4,5], and (ii) precipitation–redissolution processes using a gradient of the mobile phase made

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up of two eluents that differ significantly in their solvating strength with respect to the polymer. The use of columns packed with small pore media that cannot be permeated by the polymer molecules facilitates the separation [3,5,6].

Slow mass transfer is a serious problem in the HPLC separations of macromolecules such as proteins [7,8] and synthetic polymers using packed columns [9,10] because it contributes significantly to peak broadening. This undesired effect can be compensated partly by using very small porous beads, thus decreasing the diffusional path length [11]. Another solution is the use of non-porous particles in which diffusion within the pores cannot occur [12–14]. Small non-porous particles (1–4 μm) are preferred because only these have a sufficiently large surface area for the separation of detectable amounts of components in a sample. However, only short columns can be used due to high back pressures. Non-porous beads are frequently used for the separation of biopolymers such as proteins and nucleic acids, but they have seldom been used for the chromatography of synthetic polymers [15].

Mass transfer can be accelerated by convection. In this case the mobile phase is driven through relatively large pores within the separation medium, thus increasing the diffusivity of large molecules [16,17]. Even the convection of only a small part of the mobile phase has a large effect, as demonstrated for the very fast separation of proteins and nucleic acids in perfusion chromatography [18,19].

Total convection of the entire mobile phase is a challenging but important goal that has been achieved only with separation media with none of the interparticular voids that are so typical of particulate separation media. For example, media consisting of stacked and rolled porous sheets [20], or compressed gels [21–23] have been tested. We have developed a continuous separation medium in the shape of a rigid continuous macroporous monolith, prepared by in situ polymerization [24–29]. This novel separation medium has already shown outstanding performance in the fast separations of proteins [28,29]. In addition, we have recently shown that the monolithic column operates very efficiently in the HPLC separation of styrene oligomers, polymers, and copolymers [30].

This report provides a comparison of performance of molded monolithic macroporous poly(styrene–*co*-divinylbenzene) rod column and columns packed with non-porous and porous poly(styrene–*co*-divinylbenzene) beads in the separation of polystyrene standards.

2. Experimental

A PRP-infinity column (30×4.1 mm I.D.) packed with 4 μm non-porous polystyrene beads was purchased from Hamilton (Reno, NE, USA). Monodisperse macroporous poly(styrene–*co*-divinylbenzene) beads with a diameter of 7 μm and a median pore diameter of 143 nm as determined by mercury porosimetry were prepared by a modified procedure published elsewhere [31]. The beads were packed into a 50×8 mm I.D. stainless steel column from a slurry in tetrahydrofuran under a constant pressure of 15 MPa. The continuous monolith was prepared by polymerization of styrene and divinylbenzene at 70°C within the confines of a 50×8 mm I.D. column using a procedure described elsewhere [30]. HPLC was carried out using a Waters system consisting of two 501 HPLC pumps provided with a high pressure mixer, a 717plus autosampler, and a 486 absorbance detector. Separations at a flow-rate of 20 ml/min were carried out using an IBM LC 9560 ternary gradient liquid chromatograph provided with a low pressure mixer, and equipped with a Hewlett Packard 1050 UV detector. The data was acquired and processed with Millennium 2010 software (Waters, Milford, MA, USA).

3. Results and discussion

3.1. Column characteristics

Fig. 1 shows size-exclusion calibration curves obtained with polystyrene standards and alkylbenzenes for the three columns used in this study. The column packed with non-porous beads does not exhibit any real size-exclusion properties, and the elution of all of the standards occurs at a retention volume of 0.3 ml that equals the interparticular volume. A similarly shaped calibration curve is also found for the molded monolithic poly(styrene–*co*-

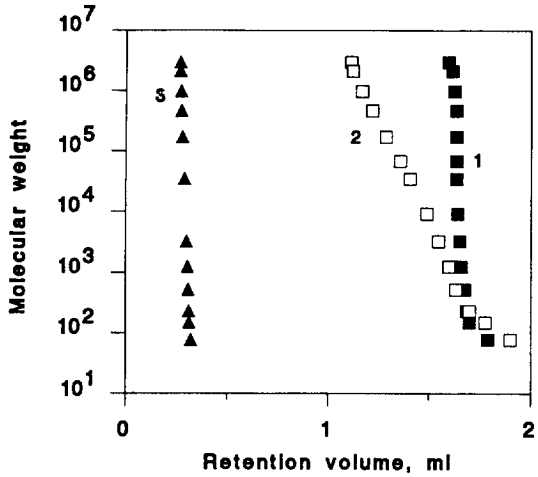

Fig. 1. Size-exclusion chromatography calibration curve of the poly(styrene–*co*-divinylbenzene) columns. Conditions: (1) rod column, 50×8 mm I.D.; (2) column packed with porous beads, 50×8 mm I.D.; (3) column packed with non-porous beads (Hamilton, PRP-infinity), 30×4.1 mm I.D.; mobile phase, tetrahydrofuran; flow-rate, 1 ml/min; analytes, polystyrene standards and alkylbenzenes; injection volume, 20 μl; UV detection, 254 nm.

divinylbenzene) rod with a pore volume of 1.6 ml. Although some differences in elution times can be observed for standards with molecular weight exceeding 2.10^6 and for molecules with molecular weights smaller than 1000, the column is not suitable for the SEC separations. All of the pores of the monolith are large and, therefore, completely permeable for standards in the molecular weight range of 10^3–10^6. In contrast, the column packed with porous beads, which has an intraparticular pore volume of 0.8 ml, shows a calibration curve that is typical of SEC columns, with an almost linear region in the molecular weight range of 10^2–10^6 well suited for the separation of macromolecules according to their size. The SEC calibration curves for both the porous beads and rod correspond well with results of mercury intrusion porosimetry The pore size distribution profile for the rod exhibits a large maximum at 7000 nm and almost no pores smaller than 700 nm. In contrast, the porous beads have pores only in the range of 10–600 nm, some of which are suitable for SEC separations.

The molded monolith has also better hydrodynamic properties than the packed columns. For example, the back pressure in the 5 cm long molded column for tetrahydrofuran at a flow-rate of 20 ml/min (55 cm/min) is only 2.6 MPa. The flow resistance, defined as a back pressure at a linear flow velocity of 1 cm/min related to 1 cm of the column length, is 9.5 kPa min cm^{-2} for the molded rod while the flow resistance for columns packed with porous and nonporous beads is 36.1 and 91.4 kPa min cm^{-2}, respectively.

3.2. Gradient separation of polystyrene standards

3.2.1. Non-porous beads

Non-porous separation media are advantageous for gradient HPLC of large molecules in adsorption modes because the restricted diffusion in pores that is characteristic of porous media is eliminated, and mass transfer is no longer a problem. Therefore, much faster separations can be achieved easily [12–14]. Fig. 2a and b show the separation of eight polystyrene standards with molecular weights ranging from 519 to 2.95×10^6. The separation, which is not very good even at a flow-rate of 2 ml/min, rapidly deteriorates at 8 ml/min. The absence of pores results in only a small surface area available for the accommodation of precipitated macromolecules [3,6,10]. As a result, co-precipitation and subsequent co-elution occur, deteriorating the separation.

3.2.2. Porous beads

In contrast to the column packed with non-porous beads, the chromatographic separation of polystyrene standards in the column packed with macroporous beads is better (Fig. 2c and d) and a good resolution can be achieved within a time period of less than 5 min. All of the standards are separated even at a flow-rate of 8 ml/min and the effect of flow-rate on the resolution is much less significant than was the case with the non-porous bead column

The chromatograms in Fig. 2c and d exhibit a large peak at the column void volume which is characteristic of pre-elution. This has also been observed by others for separations in columns packed with large pore beads [6,10]. The good solvent in which the polymer sample is dissolved and injected into the column, forms a wave that does not completely mix with the mobile phase, and passes rapidly through the column. Macromolecules that are

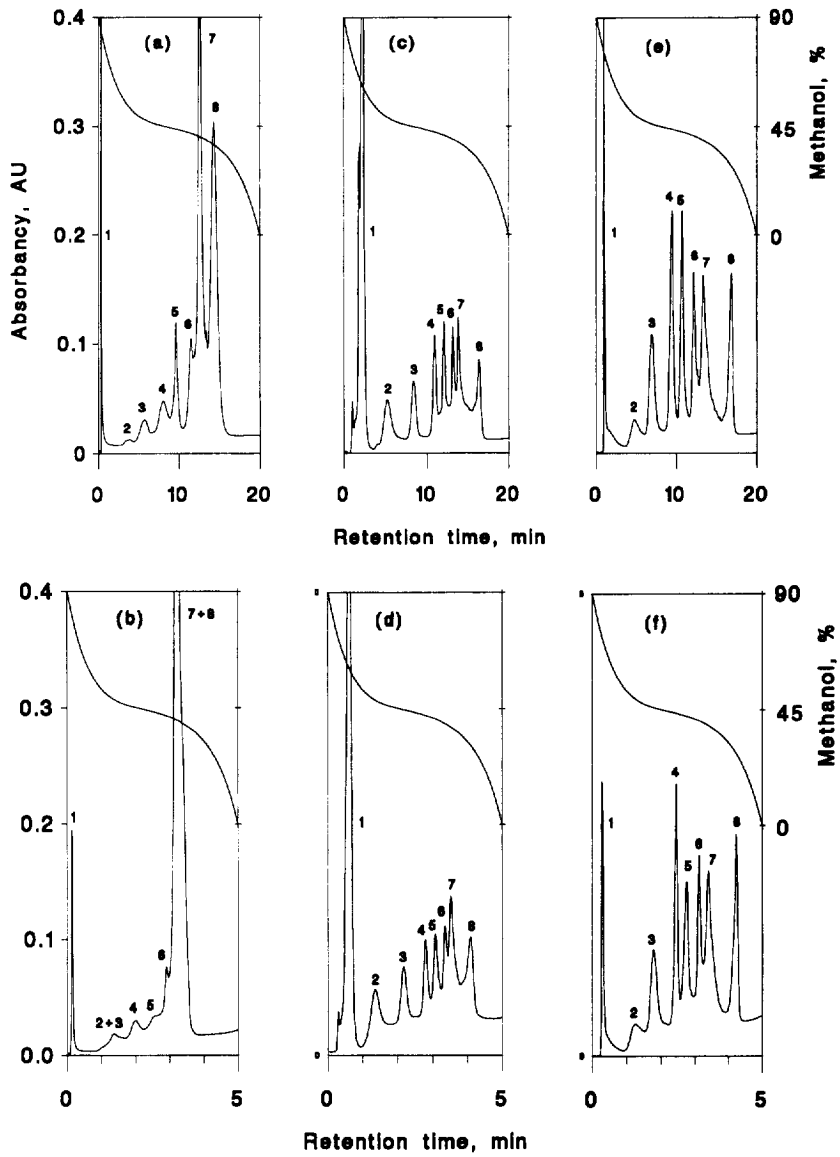

Fig. 2. Effect of a column packing on the HPLC separation of polystyrene standards. Conditions: poly(styrene–*co*-divinylbenzene) columns: 30×4.1 mm I.D., non-porous beads (a,b); 50×8 mm I.D., porous beads (c,d); 50×8 mm I.D., molded monolithic rod (e,f); mobile phase, gradient from 90 to 0% methanol in tetrahydrofuran in 20 (a,c,e) and 5 min (b,d,f), profiles superimposed in the Figures; flow-rate, 2 ml/min (a,c,e) and 8 ml/min (b,d,f); UV detection, 254 nm; analytes, polystyrenes, mol. weight 519 (1), 1250 (2), 9200 (3), 34 000 (4), 68 000 (5), 170 000 (6), 465 000 (7), and 2950 000 (8), 3 mg/ml of each standard in tetrahydrofuran; injection volume 30 μl. The peak numbers corresponds to the positions of the standards that have been injected individually.

larger than the solvent move faster than the wave as a result of size-exclusion. Therefore, a part of the sample remains dissolved in the front of the zone rich in the good solvent and elutes earlier. If this is the case, an increase in the injection volume (which translates into a larger volume of the good solvent) should result in an increase in the size of this first peak. Indeed, this is observed in our experiments and the relative area of the first peak is much larger when a volume of 100 μl is injected, instead of the typical

30 μl shown in Fig. 2, and only a small part of the sample is retained by the column.

The average retention factor in the gradient elution is directly proportional to the gradient volume, which is the product of the gradient time (steepness of the gradient) and the flow-rate. A steeper gradient accelerates the separation in comparison to a shallow gradient, but deteriorates the resolution because the peaks are eluted in a smaller volume. Therefore, the flow-rate must also be increased in order to achieve a sufficient gradient volume [9]. However, a higher flow-rate translates into higher back pressure. In contrast to the very high back pressure measured for the commercial column packed with non-porous beads, the column packed with monodisperse 7 μm beads exhibits moderate back pressure of 9.8 MPa at a flow-rate of 20 ml/min. This allows the use of steep gradients and very high flow-rates.

Fig. 3 shows fast separations of three polystyrene standards at a flow-rate of 20 ml/min using gradients

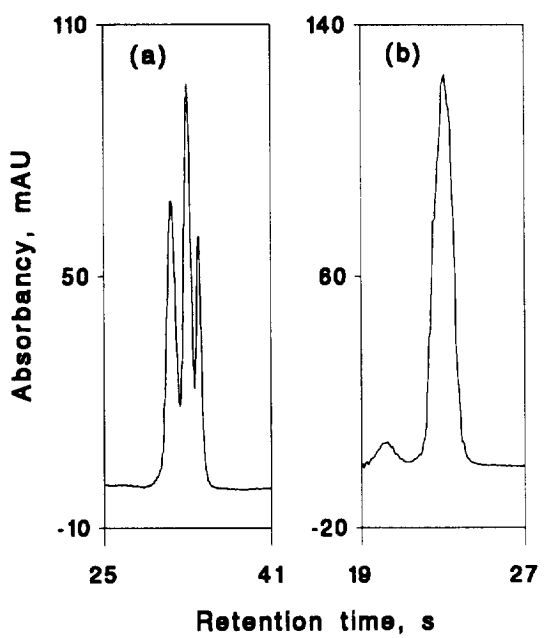

Fig. 3. Effect of gradient steepness on the very fast separation of polystyrene standards. Conditions: column, 50×8 mm I.D., porous poly(styrene–*co*-divinylbenzene) beads; mobile phase, linear gradient from 100 to 0% methanol in tetrahydrofuran within 30 (a), and 12 s (b); flow-rate, 20 ml/min; analytes, mol. weight 9200 (1), 34 000 (2), and 980 000 (3), 3 mg/ml of each standard in tetrahydrofuran; injection volume, 20 μl; UV detection, 254 nm; dead volume of the chromatographic system, 6.5 ml.

of the mobile phase that differ in their steepness. The separation is excellent at a gradient time of 30 s, and three well resolved peaks are obtained within 8 s (Fig. 3a). However, a further increase in the gradient steepness at the same flow-rate leads to rapid deterioration of the separation. There is no separation at a gradient time of only 12 s (Fig. 3b). A gradient volume of 4 ml is obviously not sufficient to separate the polystyrene standards.

3.2.3. Porous monolithic rod column

Fig. 2e and f show that an excellent separation can be achieved within a short period of time by increasing the flow-rate. For example, 16 min are needed for the separation at a flow-rate of 2 ml/min while only 4 min are sufficient for the same separation at 8 ml/min. There is almost no difference between the resolutions at both flow-rates. This result alone is not sufficient to document the significance of enhanced mass transport because the peak width even for the narrow polystyrene standards can be the result of molecular weight distribution rather than transport kinetics within the column [3]. However, we have already demonstrated for single polymer species such as proteins that mass transfer is indeed dramatically improved in the monolithic column [28]. Another advantage of the monolithic medium is the considerably reduced pre-elution that is characteristic of porous beads.

Fig. 4 shows the separations of three polystyrene standards that were carried out using very steep gradients and a flow-rate of 20 ml/min. In contrast to the column packed with porous beads, a good separation is achieved within a mere 4 s in a 12 s gradient of the mobile phase (Fig. 4b). An even faster separation (not shown here) with a mobile phase that changes from 100% methanol to 100% tetrahydrofuran in a single step (the gradient time is about 6 s) does not lead to complete separation. However, the chromatogram still exhibits two peaks and a shoulder for the smallest standard.

3.3. Separation mechanism in monolithic columns

Two different retention mechanisms are generally considered to control the chromatographic separation of high-molecular weight solutes: normal retention and precipitation–redissolution [3–6]. An extensive

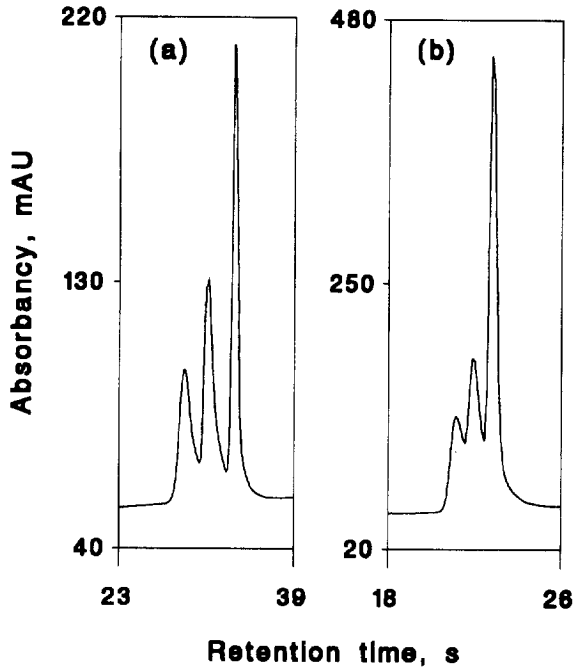

Fig. 4. Effect of gradient steepness on the very fast separation of polystyrene standards. Conditions: column: 50×8 mm I.D., molded poly(styrene–*co*-divinylbenzene) monolith; for other conditions see Fig. 3.

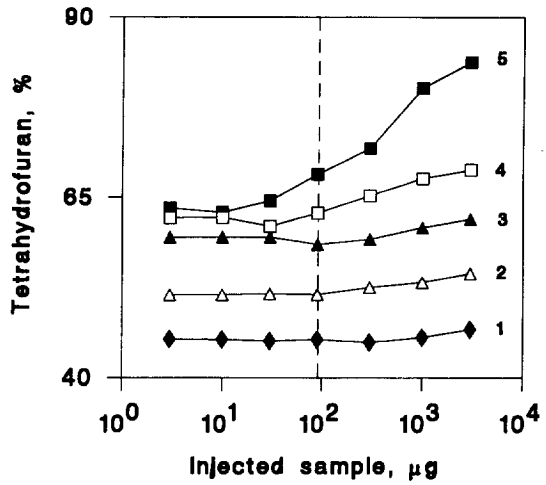

Fig. 5. Percentage of good solvent in the mobile phase required for the elution of polystyrene standards as a function of the injected amount of the sample. Conditions: poly(styrene–*co*-divinylbenzene) monolithic rod column, 50 ×8 mm I.D.; mobile phase, linear gradient from 0 to 100% (1, 2), or 30 to 100% (3, 4, 5) of tetrahydrofuran in methanol; flow-rate, 1 ml/min; detection, UV at 254 nm and evaporative light scattering at 50°C and 10 l/min air flow; analytes, polystyrenes molecular weight 9680 (1), 34 500 (2), 170 000 (3), 465 000 (4), and 2950 000 (5), 10 mg/ml in tetrahydrofuran.

study of Quarry et al. [3] suggests that the molecular weight of the sample, its solubility, and the amount injected are the most important variables in determining which retention mechanism is operative in the separation. For example, the composition of the mobile phase required for elution remains constant within a broad range of amount of sample injected for the normal retention, while the percentage of good solvent necessary for elution increases in the case of the precipitation–redissolution mechanism. Retention curves shown in Fig. 5 for polystyrene standards have a profile similar to that observed for the elution of polymers from packed C18 columns [3]. Although an increase in the percentage of THF in the mobile phase required for elution can be seen for all of the standards, this is much steeper for higher molecular weight polystyrenes suggesting that the precipitation–redissolution is involved at least for these standards.

Measurements of isocratic retention also provide insight into the retention mechanism. For normal retention, the retention time depends on the composition of the mobile phase and polymer analytes can always be eluted as a distinct near-Gaussian peak. In contrast, it is impossible to obtain elution bands at retention times exceeding the retention time of the standard under non-retained conditions in the precipitation–redissolution mode. The elution curves obtained for a polystyrene standard with a relatively low molecular weight of 9680 are shown in Fig. 6. Clearly, the retention time for the main peak remains unchanged within a range of THF content from 43 to 40%, only the peak height decreases as more molecules of the sample are retained. This indicates the strong effect of precipitation in the separation process even for this low molecular weight standard. The concentration range is relatively narrow because the k' vs. mobile phase composition curve for polymers is very steep. The sample is completely retained in the mobile phase that contains less than 40% THF and no peak can be observed while no retention is observed at a concentration exceeding 43%.

Although the adsorption mechanism of retention

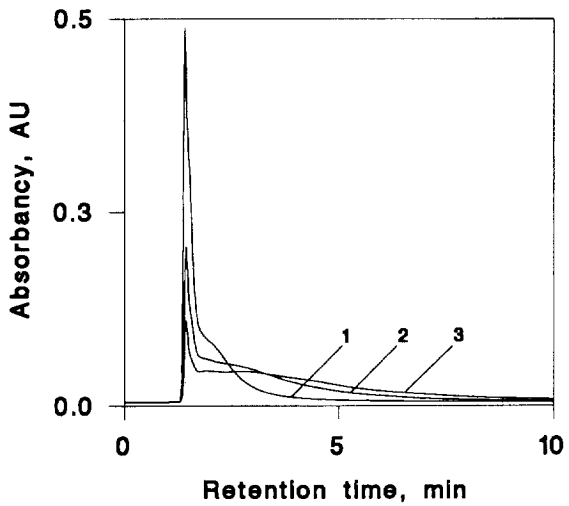

Fig. 6. Effect of mobile phase composition on the elution band of polystyrene standard with a molecular weight of 9680. Conditions: poly(styrene–*co*-divinylbenzene) monolithic rod column, 50 ×8 mm I.D.; mobile phase, mixture of tetrahydrofuran and methanol 43:57 (1), 41:59 (2), and 40:60 (3), vol%; flow-rate, 1 ml/min; UV detection, 254 nm; analyte, sample concentration, 10 mg/ml in tetrahydrofuran; injection volume, 9 μl.

cannot be excluded completely, the experimental data suggests that the precipitation–redissolution effects are quite strong and contribute considerably to the retention of synthetic polymers in the monolithic column. This is not completely unexpected because this column only has a limited surface area in the large pores available for the interactions [32] and, in contrast to some of the silica based separation media, this column also contains very small pores that are not permeated by polymer solutes. Therefore, these solutes migrate during the gradient elution through the column faster than the mobile phase zone capable of dissolving them. As a result, the polymer molecules precipitate as they move from the zone of stronger solvent into the poorer solvent and dissolve only when the solvent strength is again sufficient to dissolve them.

4. Conclusion

The commercial column packed with small nonporous poly(styrene–*co*-divinylbenzene) beads is not ideal for the HPLC separation of polystyrene standards even at modest flow-rates. Higher flow-rates are prohibited for this column due to a sharp increase in the back pressure. In contrast, larger macroporous beads and the molded monolithic rod column, exhibit tolerable back pressures in the range of flow-rates up to 20 ml/min. Both the column packed with macroporous beads and the molded rod column show good resolution, provided a sufficiently large gradient volume is used. Although good separations of polystyrene standards are achieved with both columns, the undesired "pre-elution" is generally lower in the molded column. The absence of both co-precipitation and pre-elution is an important advantage of the monolithic columns, particularly for separations in which a large volume of sample solution must be injected.

Low back pressures allow separations to be carried out at very high flow-rates and in very steep gradients. A decrease in the gradient volume leads to a rapid loss of separation ability of the packed column. In contrast, the monolithic column retains its separation properties, even at small gradient volumes, and the separations can be achieved within a few seconds. This indicates that the unique porous properties of the molded monolithic column are particularly well suited to the very fast separations needed for the routine quality control and real-time direct monitoring of chemical processes.

Though this report describes the separation of polystyrene standards, the short molded rod can be used for the fast separation of many other polymers using a suitable pairs of solvents and non-solvents. The molded separation media can also be used for the analysis of copolymers in which, in addition to molecular weight, the composition of the copolymer itself contributes to the retention.

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