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Preparation of Porous Poly(styrene-*co*-divinylbenzene) Monoliths with Controlled Pore Size Distributions Initiated by Stable Free Radicals and Their Pore Surface Functionalization by Grafting

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ABSTRACT: A stable free radical (SFR) mediated preparation of porous poly(styrene-*co*-divinylbenzene) monoliths using new types of SFRs and a novel binary porogenic solvent consisting of poly(ethylene glycol) and 1-decanol has been studied. Polymerizations carried out in the presence of 3-carboxy-2,2,5,5-tetramethylpyrrolidinyl-1-oxy (3-carboxy-PROXYL) or 4-carboxy-2,2,6,6-tetramethylpiperidinyl-1-oxy (4-carboxy-TEMPO) were faster, and higher degrees of monomer conversions were achieved in a shorter period of time compared to the corresponding reactions mediated by 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO). The effect of the type of SFR (TEMPO, 3-carboxy-PROXYL, 4-carboxy-TEMPO, 4-amino-TEMPO, 4-acetamido-TEMPO, and 4-trimethylammonio-TEMPO iodide) on the pore size distribution was also investigated. The use of carboxy functional SFRs simultaneously accelerated the reaction kinetics, improved the permeability of the prepared monoliths, and enabled control of the porous properties of the monolithic polymers over a wide range simply by modifying the ratio of poly(ethylene glycol) 400 and 1-decanol in the porogenic mixture. The reinitiation capability of poly(styrene-*co*-divinylbenzene) monoliths capped with 3-carboxy-PROXYL or 4-carboxy-TEMPO moieties was utilized to perform in situ grafting of 2-hydroxyethyl methacrylate and 3-sulfopropyl methacrylate, resulting in monoliths with altered surface polarities.

Introduction

Stable free radical (SFR) mediated "living" polymerizations have been used in various branches of polymer chemistry during the past decade.[1] A series of papers report on the synthesis of linear polymers with well-defined molecular weights, the preparation of graft[2] or block[3] copolymers, and polymerizations in emulsion[4] or dispersion.[5] Most of these reports are exploiting the common features of SFR-mediated polymerization systems, which have controlled rate of monomer incorporation into the growing polymer chain, a minimum of termination reactions compared to traditional free radical polymerization, and control of end group functionality or molecular shape and size.

We have recently reported that porous poly(styrene-*co*-divinylbenzene) [poly(S-DVB)] monoliths can be prepared in the presence of the stable nitroxide radical 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO) by a mold polymerization process including the mono- and divinylic monomers as well as a porogenic solvent and a conventional free radical polymerization initiator.[6] The pore size distribution of these monolithic polymers differs fundamentally from the typical bimodal pore size distribution of poly(S-*co*-DVB) monoliths prepared without an SFR in the polymerization mixture.[7] For example, the specific surface areas of these monoliths were over 300 m^2/g, more than 1 order of magnitude larger than that of typical monoliths prepared using "traditional" mold polymerizations without adding TEMPO and polymerized at temperatures of 55−80 °C. In contrast to these "classical" monoliths, the pore size distribution for SFR-controlled materials ranged from more than 1000 nm to less than 10 nm, which rendered them more capable of separating polymers according to their molecular weight in size exclusion chromatography (SEC). We attributed this porosity to be mainly due to the high temperatures exceeding 120 °C that were required to achieve polymerization in the presence of TEMPO. We also reported the successful use of the TEMPO radical, reversibly trapped during the mold polymerization, for grafting the pore surface with various monomers.[7]

Despite these promising characteristics, our initial study of stable free radical mediated polymerization in closed molds leading to porous objects yielded polymers with a high flow impedance. However, good permeability is essential for chromatographic and other flow-through operations. Therefore, the high flow resistance made the monolithic columns prepared with TEMPO unsuitable for a number of applications. Obviously, SFR mediated polymerization at a lower temperature would be more convenient to implement, and as an additional benefit, material with large pores can be created. In our recent work,[8] we used of 2,2,5-trimethyl-3-(1-phenylethoxy)-4-phenyl-3-azahexane as an SFR initiator. This alkoxyamine was specifically designed for polymerization at considerably lower temperatures than those required for TEMPO mediated systems.[9] Indeed, monolithic materials were already formed at temperatures as low as 100 °C. However, polymerizations using this more reactive radical remained rather slow and long reaction times were required to achieve high conversions.

At this point, finding systems polymerizing faster, requiring lower polymerization temperatures, and affording monoliths with favorable porosity appeared to be instrumental for the preparation of materials with the desired properties. This paper reports the prepara-

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Scheme 1. Structures of Stable Free Radicals Used as Mediators in the Polymerization of Poly(S-*co*-DVB) Monoliths

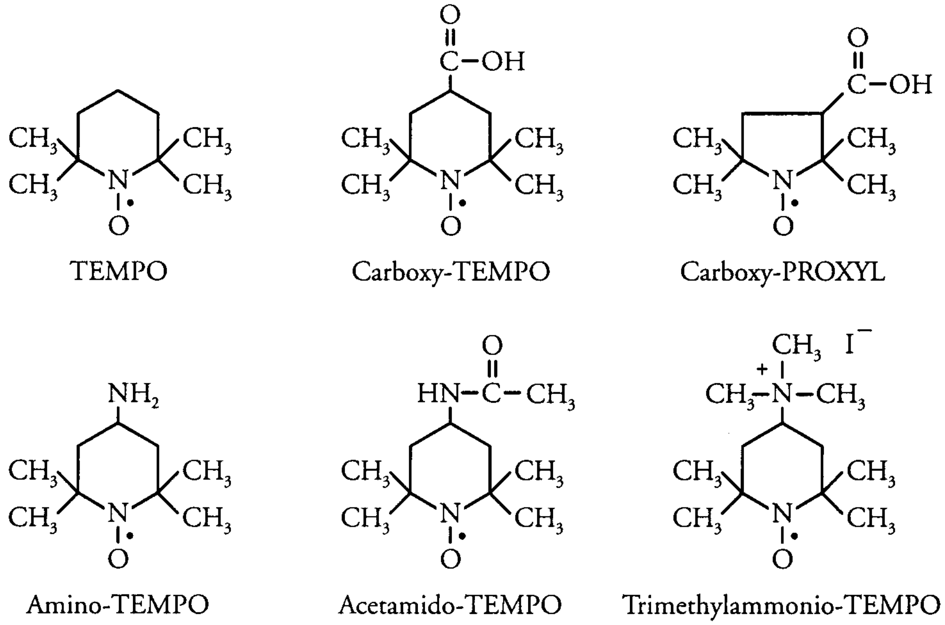

Table 1. Surface Areas and Flow Permeabilities of Poly(S-*co*-DVB) Monoliths[a]

polymer exp no.	SFR[b] (mol/mol)	PEG:1-decanol[c] (w/w)	surface area[d] (m^2/g)	flow permeability[e] (MPa (min)/mL)
1	no SFR	85:15	7.0 ± 0.2	0.5
2	TEMPO	85:15	1.2 ± 0.1	0.1
3	3-carboxy-TEMPO	85:15	146.6 ± 0.6	12.1
4	3-carboxy-PROXYL	85:15	72.1 ± 0.1	8.6
5	3-carboxy-PROXYL	75:25	183.0 ± 3.0	9.7
6	3-carboxy-PROXYL	95:05	2.6 ± 0.0	0.2

[a] The polymerization mixture contained 50% (w/w) monomers (styrene and divinylbenzene; 1:1) and 50% porogenic solvent (PEG 400 and 1-decanol), and the polymerization was carried out in 50 mm long by 8 mm i.d. columns at 130 °C for 10 h. [b] The molar BPO:SFR ratio was kept at 1:1.3, except for a reference polymer that was polymerized without SFR. [c] Weight ratio between PEG 400 and 1-decanol in the polymerization mixture. [d] Surface area determined from the desorption curve for nitrogen. [e] Flow permeability as determined by the slope of the back-pressure curve when THF was pumped through the polymer.

tion of highly permeable poly(S-DVB) monoliths suitable for flow-through operations utilizing polymerizations mediated by commercially available and stable free radicals, 3-carboxy-PROXYL and 4-carboxy-TEMPO. Other commercially available and stable free radicals (see Scheme 1) have also been tested. Among these, 3-carboxy-PROXYL and 4-carboxy-TEMPO were advantageous since they accelerated the kinetics of the polymerization process considerably and allowed the capped radicals located at the surface of pores to be used for grafting hydrophilic monomers.

Experimental Section

Chemicals. The monomers styrene (99%; Aldrich, Steinheim, Germany) and divinylbenzene (DVB, 70−85% mixtures of isomers; Fluka, Buchs, Switzerland) were freed from polymerization inhibitors using basic Al_2O_3 ($\approx$0.1 g/mL monomer; Brockmann *I*, standard grade; Aldrich) stirred by shaking for at least 24 h. 2-Hydroxyethyl methacrylate (HEMA, 95%; Fluka) and 3-sulfopropyl methacrylate (98%, Aldrich) were used as received. Benzoyl peroxide (BPO, 97%), 3-carboxy-2,2,5,5-tetramethylpyrrolidinyl-1-oxy (3-carboxy-PROXYL, >99%), 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO, 99%), 4-carboxy-2,2,6,6-tetramethylpiperidinyl-1-oxy (4-carboxy-TEMPO, 97%), 4-amino-2,2,6,6-tetramethylpiperidinyl-1-oxy (4-amino-TEMPO, 97%), and 4-acetamido-2,2,6,6-tetramethylpiperidinyl-1-oxy (4-acetamido-TEMPO, 98%) were purchased from Aldrich and used without further purification. 4-Trimethylammonium-2,2,6,6-tetramethylpiperidin-1-oxy iodide (4-trimethylammonio-TEMPO iodide, purity data not available) was purchased from Molecular Probes (Eugene, OR). Poly(ethylene glycol) (PEG 400) and 1-decanol (99%) were obtained from Aldrich.

Preparation of Polymers. The polymer monoliths were generally prepared by dissolving the initiator (BPO) and the SFR in a solution containing divinylbenzene and styrene at 1:1 weight ratio and porogen, which was a binary mixture of varying amounts of PEG 400 and 1-decanol. For example, the polymerization solution corresponding to experiment 4 in Table 1 contained 7.5 mg (0.031 mmol) of BPO, 7.5 mg (0.040 mmol) of carboxy-PROXYL, 0.75 g (7.20 mmol) of styrene, 0.75 g (5.76 mmol) of DVB, 1.275 g ($\sim$3.19 mmol) of PEG 400, and 0.225 g (1.42 mmol) of 1-decanol. After sonication (Bransonic 221) for 10 min and purging with helium for 10 min, 50 × 8 mm i.d. or 50 × 4 mm i.d. stainless steel columns were filled with the solutions, sealed using stainless steel fittings and PTFE gaskets in order to prevent any leakage during the polymerization, and placed vertically in a stirred thermostated oil bath. The polymerizations were carried out at a temperature of 130 °C for 10 h, except for polymers prepared in the conversion experiments described below.

Size Exclusion Chromatography. After the polymerizations had been completed, the columns were equipped with stainless steel frits and HPLC fittings. The soluble compounds remaining in the monolith's pore structure were removed by washing with at least 100 mL of THF pumped through at a flow rate of 0.2 mL/min. The stainless steel columns (50 × 8 mm i.d.) were then connected to an HPLC system and equilibrated at 0.5 mL/min of THF, using a Bischoff (Leonberg, Germany) HPLC compact pump. The SEC calibration curves were determined for the monoliths using polystyrene standards (1 mg/mL, Polymer Laboratories, Church Stretton, UK), having molecular weights 580−3 220 000, and THF as the mobile phase. The solutions were injected through a Rheodyne (Cotati, CA) model 7125 loop injector with a 20 μL stainless steel injection loop. Ultraviolet detection was carried out at 254 nm, and the detector signal was recorded on a Hewlett-Packard (Palo Alto, CA) model 3396A integrator. The retention volumes for polystyrene standards were normalized taking the retention volume for polystyrene with $M_w = 580$ as a base. All SEC experiments were carried out at room temperature (~22 °C). Separation of a polystyrene mixture containing 1 mg/mL with respect to each polystyrene standard (molecular weights: 580, 210 500, and 3 200 000) was carried out using THF as eluent at a flow rate of 1.0 mL/min.

Scanning Electron Microscopy. A piece of monolithic polymer was snapped apart and placed on sticky carbon foil, which was attached to a standard aluminum specimen stub. The polymer was coated with about 20 nm of gold by sputter coating (Edwards S150A sputter coating unit, Edwards high vacuum, incorporating an automatic tilting and rotation device). Microscopic analysis was carried out in an S-360 iXP SEM (Leo Electron Microscopy Ltd., Cambridge, UK) operated in LaB_6 mode, 10 kV, 100 pA probe current, and 0° tilt angle.

Surface Area Analysis. The polymer samples were dried at 80 °C for 48 h and cut into cubiform pieces with approximately 2−3 mm sides. The surface area was calculated from the desorption isotherm of N_2 using a Micromeritics (Norcross, GA) FlowSorb II 2300 instrument. Three surface area measurements were carried out for each polymer, and the values are presented as mean ± standard deviation.

Electron Spin Resonance (ESR). After the chromatographic evaluation of the monoliths had been completed, the polymeric material was removed from the columns and ground to powder. The ground polymers were dried at room temperature for about 24 h and weighed into NMR tubes (≈11 mg of polymer/tube). Spectra were acquired using a Bruker (Rheinstetten, Germany) ESP 300-E ESR spectrometer fitted with a variable temperature control unit. Typically, the modulation amplitude was set to 1.0, and the modulation frequency was kept at 100 kHz. Duplicate scans were run for each time point. Spectra corresponding to $t = 0$ were measured at room temperature (298 K). The sample tubes were then removed from the spectrometer while the cavity was heated to the desired temperature. When this temperature was reached, the sample was again fixed in the spectrometer cavity, and ESR spectra were collected at regular intervals. The release of 3-carboxy-PROXYL radical was monitored, and the relative concentration of radicals at each temperature was determined by double integration of the ESR spectra. The initial, linear part of the signal for 3-carboxy-PROXYL release was used to determine the activation energy of the polymer−SFR bond cleavage. The ESR spectra were acquired with the materials suspended in xylene. Additional spectra from the release of 3-carboxy-PROXYL at 398 K were also obtained for the polymers in the dry state (Figure 2).

The stability of 3-carboxy-PROXYL in the porogen mixture was assessed by adding 1.2 mg of 3-carboxy-PROXYL to a mixture containing 400 mg of PEG and 100 mg of 1-decanol. After mixing, the sample was heated to 398 K in the ESR spectrometer cavity. Spectra were collected continuously in order to monitor changes as a result of possible side reactions of the SFR.

Grafting Procedure. Grafting of HEMA onto a monolith prepared using a polymerization mixture containing 6.8 mg (0.036 mmol) of carboxy-PROXYL, 8 mg (0.033 mmol) of BPO, 0.80 g (7.68 mmol) of styrene, 0.80 g (6.14 mmol) of DVB, 1.92 g (~4.8 mmol) of PEG, and 0.48 g (3.03 mmol) of 1-decanol was carried out using a stock solution prepared from 0.62 g of HEMA and 5.52 g of cyclohexanol. No initiator was added to this solution. The grafting solution was pumped into the column through a 3 mL loop at room temperature at a flow rate of 0.2 mL/min. The grafting reaction was allowed to proceed for 12 h at 130 °C; the column was cooled to room temperature, connected to an HPLC system, and washed with methanol/water 80/20 (v/v). The wettability of grafted and ungrafted samples by distilled water was estimated visually with the ground powders. Grafting of poly(3-sulfopropyl methacrylate) was carried out using the same procedure and monoliths as for the HEMA grafting experiments, using a solution of 1.75 g (7.10 mmol) of 3-sulfopropyl methacrylate dissolved in 10 mL of DMSO and reacted at 130 °C for 8 h.

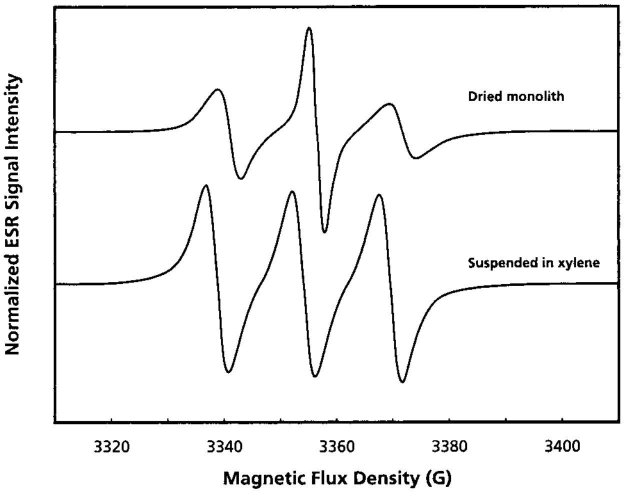

Figure 1. Electron spin resonance spectra of a poly(S-*co*-DVB) monolith sample polymerized in the presence of 3-carboxy-PROXYL after heating in the ESR cavity for 20 min at 398 K. Experiments were carried out with the monolith in the dry state and when suspended in xylene.

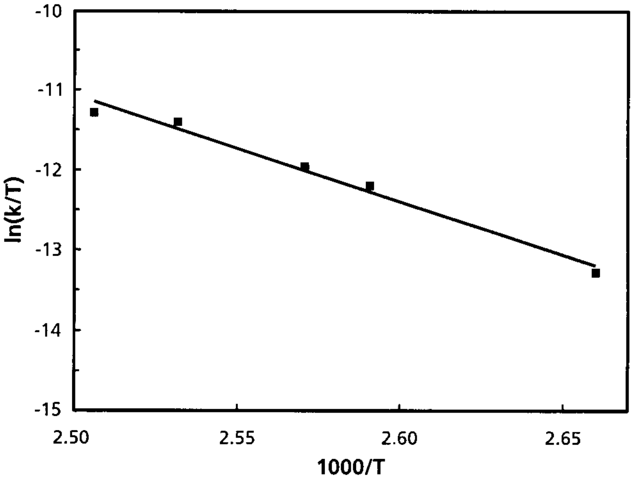

Figure 2. Arrhenius plot based on the initial rate of radical release at different temperatures from poly(S-*co*-DVB) synthesized with a BPO:3-carboxy-PROXYL molar ratio 1:1.3.

Determination of Polymerization Kinetics. The conversion was monitored as a function of polymerization time for a polymerization mixture containing 25% styrene, 25% divinylbenzene, 40% PEG, and 10% 1-decanol at a temperature of 130 °C. The amount of BPO was kept at 0.5% with respect to the weight of monomers and the molar ratios BPO:3-carboxy-PROXYL at 1:1.3 and 1:3, respectively. The polymerization mixture was transferred to 42 mm high by 16 mm i.d. glass vials (~ 2 g each), which were sealed and immersed into the oil bath at 130 °C. At different conversion time points, the corresponding vials were removed from the oil bath, and the polymers were transferred to a glass filter. After grinding the polymers to powder, they were carefully washed with large amounts of methanol and acetone and dried at 80 °C to constant weight. The conversion was calculated from the weight of the dry polymers relative to the weight of the monomer phase in the polymerization solution. Analogous experiments were also carried out in the presence of 4-carboxy-

TEMPO and TEMPO using a molar ratio of BPO to SFR of 1:1.3.

Evaluation of Grafting. The chromatographic properties of the monolithic poly(S-DVB) column in the reversed phase mode before and after grafting with HEMA were evaluated in an isocratic operation using a mixture of methanol and water (80/20 v/v) as an eluent at a flow rate of 0.5 mL/min. A homologous series of methyl, ethyl, propyl, and butyl benzoate were injected through the 20 μL loop and detected at 254 nm. The retention factors k' were calculated using the following equation

$$k' = (t_{\mathrm{r}} - t_{\mathrm{m}})/t_{\mathrm{m}}$$

where t_{r} and t_{m} are the retention times of the benzoate ester and nitrate ion, respectively. The contents of strong cation exchange functionalities in poly(S-DVB) monoliths grafted with 3-sulfopropyl methacrylate were determined by magnesium ion uptake capacity. The column was equilibrated with a solution of 100 mmol/L $MgCl_2$ and then washed with deionized water. The Mg^{2+} ions were eluted with 100 mmol/L $CaCl_2$ and subsequently determined by atomic absorption spectrometry using a Varian (Walnut Creek, CA) AA-875 spectrometer.

Ion Exchange Separation of Proteins on Grafted Cation Exchange Monolith. The poly(S-*co*-DVB) monolith (50 × 4 mm i.d.) grafted with 3-sulfopropyl methacrylate according to the procedure above was equilibrated with 0.05 M NaCl in 4 mM phosphate buffer, pH 7. Onto this column was injected a 20 μL aliquot of a mixture of protein probes (0.5 mg/mL each of myoglobin, cytochrome *c*, and lysozyme; all from Sigma, St. Louis, MO), and elution was accomplished by a linear gradient ranging from the starting buffer to a final buffer composition of 0.25 M NaCl in 20 mM phosphate buffer pH 7 at a flow rate of 1.0 mL/min. Detection was by UV spectrophotometry at 280 nm.

Results and Discussion

The objective of this study was to develop a method for the preparation of porous monoliths by polymerization mediated by SFR with vastly accelerated polymerization kinetics as compared to the TEMPO mediated system, which we reported recently.[6] A number of papers have demonstrated that SFR mediated radical polymerizations are considerably slower than traditional radical polymerizations.[1,6,9,10] This also applies to TEMPO mediated bulk polymerizations resulting in porous monoliths,[6] since the conversion was only about 85% after 48 h at 130 °C using a BPO:TEMPO ratio of 1:1.2. The reaction rate was improved for polymerizations in the presence of acylating reagents such as acetic anhydride.[6,11−13] Veregin et al.[14] reported 3-carboxy-PROXYL as an alternative to TEMPO to mediate SFR polymerization of styrene and yield polymers with low polydispersity. They estimated the energy required to break the labile bond in the reaction product of SFR with a single styrene molecule to be 113 kJ/mol for 3-carboxy-PROXYL and 129 kJ/mol for TEMPO. Although these values relate to model compounds comprised of only a single monomer unit, they indicated that 3-carboxy-PROXYL could be a better choice for the preparation of monoliths when more rapid polymerization kinetics are sought.

To investigate the suitability of 3-carboxy-PROXYL for the preparation of porous monoliths, ESR measurements were carried out with poly(S-DVB) samples prepared using a BPO:3-carboxy-PROXYL molar ratio of 1:1.3. Despite the washing step, low concentrations of unattached free radicals trapped in the polymer matrix yield a low background signal in the ESR cavity at room temperature. However, after heating the polymer samples to sufficiently high temperatures, the signal from carboxy-PROXYL increased substantially, and we therefore conclude that the free radicals monitored while the polymers are heated in the ESR cavity (Figure 1) are released from the polymer terminals. The ESR spectra thus correspond to the free nitroxide radicals present in the pores or in the xylene solution. Initially, a linear radical release curve is obtained from poly(S-DVB) samples prepared using carboxy-PROXYL, whereafter the curve levels off and reaches a plateau. If the sample is allowed to reattain room temperature, the free radical concentration will remain essentially at the plateau level reached during heating, which indicates that recombination is restricted. Quantifying the amount of free radicals with ESR is difficult, but a significant amount of SFR was found in the xylene after the polymer was filtered off. The activation energy required to break the bond between 3-carboxy-PROXYL and the cross-linked poly(S-DVB) monoliths was estimated from Arrhenius plots of the initial release rate of free radicals at different temperatures. Although ESR spectra for polymeric matrixes often suffer from anisotropic effects and poor line shapes that may make quantitative measurements difficult,[15] we were able to achieve a release of free radicals from the polymer on heating and determined the activation energy to be about 113 kJ/mol (Figure 2). This value closely matches the previously reported value for the styrene-1-mer.[14] Shapes of the ESR spectra for a typical monolith sample prepared with 3-carboxy-PROXYL are shown in Figure 1, both in the dry state and suspended in xylene. The line shape of the sample suspended in xylene is characteristic of a rapidly tumbling nitroxide radical in solution, whereas the spectrum acquired in the dry state shows significant anisotropy. The SFR is thus released directly into xylene solution or at least to a highly swollen part of the system where xylene has relatively unrestricted access. Since the molecular sizes of the graft monomers are similar to xylene, it is reasonable to assume that these monomers will have access to the free radical sites generated at the polymer surface.

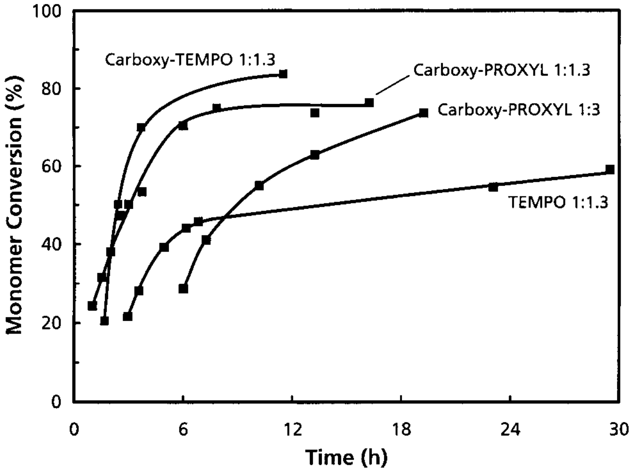

Figure 3. Conversion of styrene and divinylbenzene at 130 °C in the presence of TEMPO and 4-carboxy-TEMPO at a molar ratio of 1:1.3 and 3-carboxy-PROXYL at two different molar ratios.

The polymerization kinetics of SFR mediated polymerization at 130 °C leading to a monolith is presented in Figure 3. At a BPO:3-carboxy-PROXYL ratio of 1:1.3, the conversion reached more than 75% after already 16 h. In contrast, less than 60% conversion was achieved after 30 h reaction in the presence of TEMPO at the same molar ratio. The conversion was 74% after 20 h of reaction even for polymerization of mixtures containing BPO and 3-carboxy-PROXYL in an extreme ratio of 1:3. Such high levels of TEMPO could not be used without addition of an acylating agent, since the polymerization rate decreases rapidly as demonstrated by precipitation in the polymerization mold.[6] The polymerization kinetics is similar for both 4-carboxy-TEMPO and 3-carboxy-PROXYL (Figure 3).

Porous Properties. Our previous work demonstrated the control of porous properties of poly(S-DVB) and poly(2,3-epoxypropyl methacrylate-*co*-ethylene dimethacrylate) monoliths during their preparation by varying the polymerization temperature, the thermodynamic quality of the porogenic solvent, and the cross-linking density.[7] For example, the pore size distribution profile can be shifted toward smaller pores by increasing the polymerization temperature or by using a porogenic solvent with better solubilizing power for the polymer. These reaction parameters affecting the pore formation process were operational in a number of systems including photopolymerization[16] and enabled the preparation of monoliths with porous properties optimized for the desired flow-through applications. However, as we started to study SFR mediated bulk polymerizations, we realized that the rules established for radical polymerizations run at temperatures of 55−90 °C could not be extrapolated to these new polymerizations requiring much higher temperatures. For example, a considerably different pore size distribution was observed for monoliths prepared at a temperature of 130 °C, whether or not TEMPO was added.[6] Although the SFR mediated process allowed control of the polymerization and enabled surface initiated grafting, the pore size distribution required for easy flow-through was difficult to obtain. Despite some progress using the new alkoxyamine initiators[8,9] and polymerizations at lower temperatures, the desired pore size distribution was not accessible. Therefore, we tested the other option, the use of specifically designed porogenic solvent mixtures. In the search for such a system, we found that poly(ethylene glycol) (PEG) with a molecular weight of 400 in a mixture with long chain alcohol such as 1-decanol represents a porogen of choice. Polymers are powerful porogens affording typically macroporous polymers with larger pores and poly(ethylene glycols) were used for the preparation of porous methacrylamide based cross-linked polymers.[17]

Since the monolithic materials are mostly used in combination with liquids, determination of their porous properties in the solvated state is more valuable than typical methods implemented in the dry state such as mercury intrusion porosimetry. One of these techniques is SEC, which is well-suited for monoliths in the column format. The slope of the SEC calibration curve provides information related to the practical pore size distribution. The preparation of poly(S-DVB) monoliths using conventional polymerizations often affords materials unable to separate polymers according to their molecular weight. This means that the porous structure with most of the pore volume accounted for by pores with diameters around 1000 nm does not discriminate the polymer molecules with respect to their size.

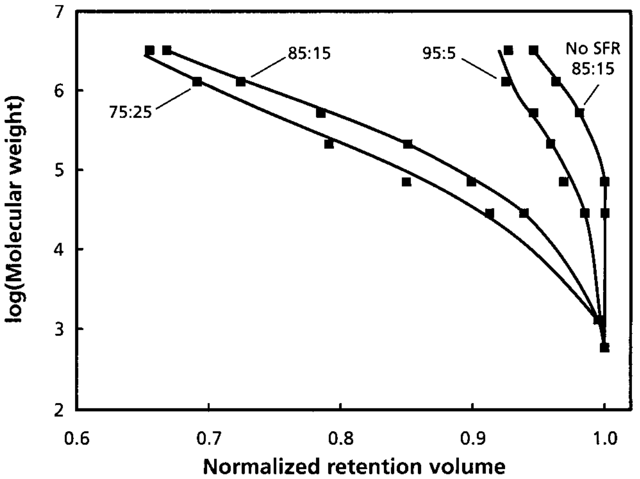

Figure 4. SEC calibration curves of polystyrene for poly(S-*co*-DVB) monoliths (50 mm by 8 mm i.d.) polymerized at 130 °C with different PEG:1-decanol ratios, using 3-carboxy-PROXYL as the SFR at 1:1.3 molar ratio. A reference sample polymerized without SFR at a PEG:1-decanol ratio of 85:15 is also included.

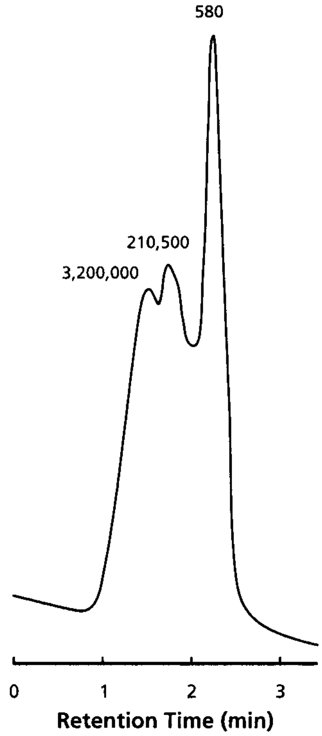

Figure 5. SEC separation profile of a 20 μL sample of a polystyrene mixture (1.0 mg/mL with respect to each PS) at the molecular weights indicated, using a poly(S-*co*-DVB) monolith (50 mm by 8 mm i.d.) prepared using the composition described in Table 1, experiment 4.

Figure 4 shows the SEC calibration curves for poly(S-DVB) monoliths prepared by SFR mediated polymerization using various compositions of the porogen phase. Changes in the ratio between the porogen components enabled fine-tuning of the pore size distribution. The SEC curves show that monoliths containing mainly larger pores were obtained with porogenic mixtures consisting of higher proportion of PEG. These columns do not appreciably separate polymers according to their molecular weight. However, an increased proportion of 1-decanol in the porogen mixture significantly improved the separation capability and polymer standards with molecular weights ranging from 580 to 3 220 000 were well separated. The difference in retention volumes monitored for the smallest and largest polymer standard was 0.73 mL. Taking into account the

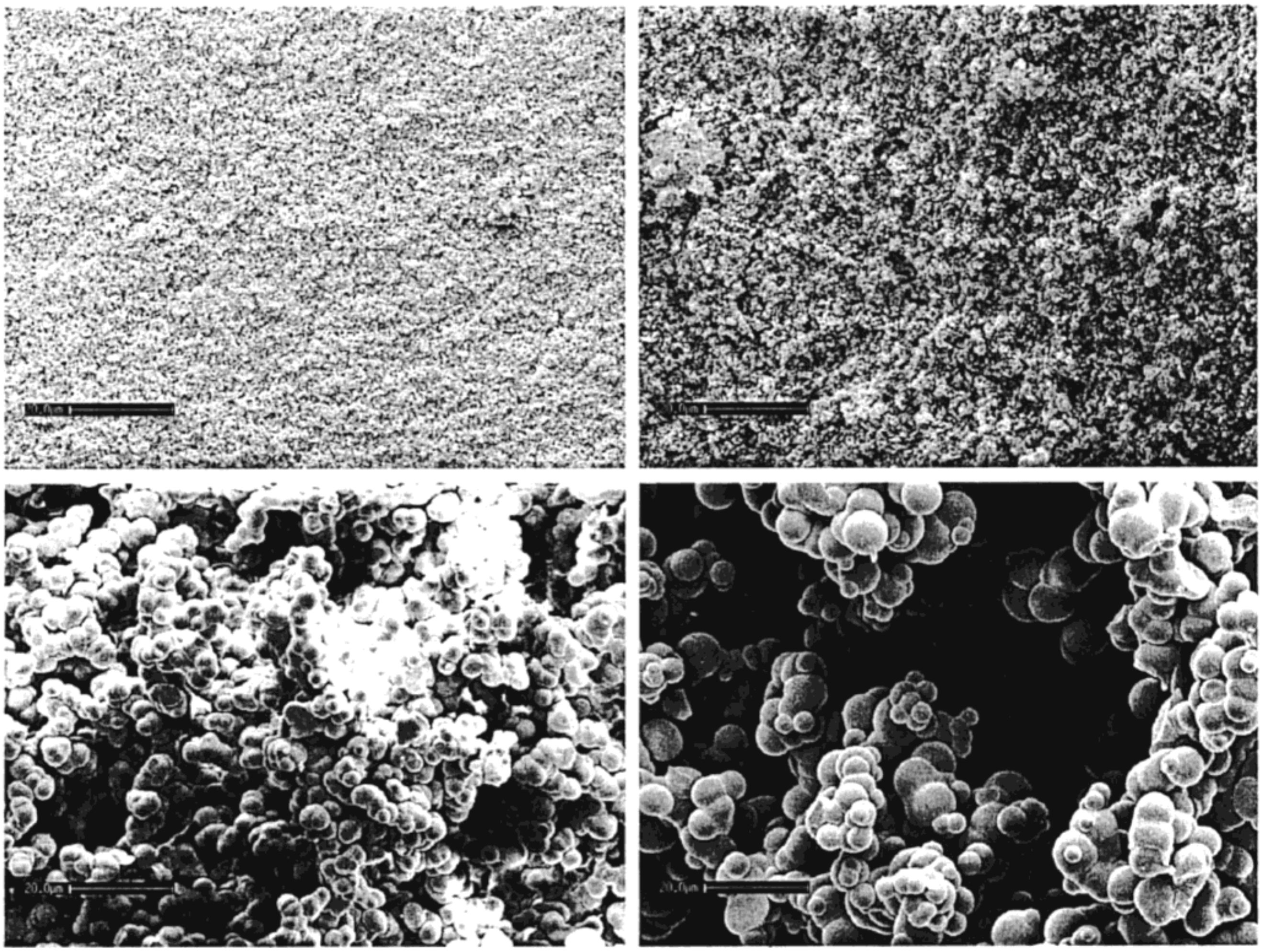

Figure 6. SEM micrographs of poly(S-*co*-DVB) monoliths polymerized at 130 °C with PEG:1-decanol ratios of 75:25 (a, upper left), 85:15 (b, upper right), and 95:5 (c, lower left). A reference sample polymerized with TEMPO instead of 3-carboxy-PROXYL (d; PEG:1-decanol ratio of 85:15) is shown in the lower right micrograph.

column size of 50 × 8 mm i.d., this volume translates to about 29% of the total column volume (2.5 mL). Since this column was prepared from a polymerization mixture containing 50% of monomers, this retention volume also means that at least 60% of the theoretical pore volume was accessible to the standards. Figure 5 demonstrates the separation of a mixture of three polystyrene standards with molecular weights of 3.22 $\times 10^6$, 2.105 $\times 10^5$, and 5.8 $\times 10^2$ using the 50 mm long poly(S-DVB) monolith. Although the column efficiency for this unusually short SEC column was only modest, its selectivity was good. Considering the very low back-pressure experienced in this SEC separation (cf. Table 1), the length of the columns can be easily extended for more efficient separations. Figure 6 shows SEM micrographs of porous monoliths prepared in the presence of a porogen with different ratios of PEG and 1-decanol, the SEC calibration curves of which were presented in Figure 4. The micrographs clearly visualize the striking effect of the porogen composition on the porosity. At a PEG:1-decanol ratio of 95:5 (Figure 6c) the SEM picture shows an open structure with large pores, which correlates well to the lack of size exclusion separation capability for this polymer (Figure 4). When the PEG to 1-decanol ratio was decreased, the SEM pictures indicate that the large pore channels were replaced with more compact structures (Figure 6a,b). As discussed above, these monoliths possess a broad size exclusion separation range, evident from their SEC calibration curves (Figure 4).

Nitrogen adsorption/desorption measurements is typically used to determine the specific surface area. Since the major contribution to the surface area of macroporous polymers comes from pores smaller than 10 nm, these data provide additional information characterizing the porous properties. Table 1 shows that the surface area increased dramatically from 2.6 to 182.9 m^2/g by changing the PEG:decanol ratio from 95:5 to 75:25. Surface areas similar to these were also obtained in our previous studies concerning the preparation of poly(S-DVB) monoliths using both standard low-temperature and TEMPO moderated high-temperature polymerizations. However, monolithic columns featuring high surface areas always exhibited excessively high back-pressures. In contrast, Table 1 shows that a higher percentage of PEG in the porogenic mixture enabled the preparation of monolithic columns with excellent permeability. The SEM micrographs confirm the presence of large transecting pores in monoliths prepared from mixtures with higher percentage of PEG. Owing to the advantageous porous structure, the back-pressures of these columns were well within the operating range of our chromatographic equipment even for polymers with the highest surface area. Obviously, the use of tailored porogenic solvent enabled us to resolve the “conflicting” demand for high surface area and low flow resistance.

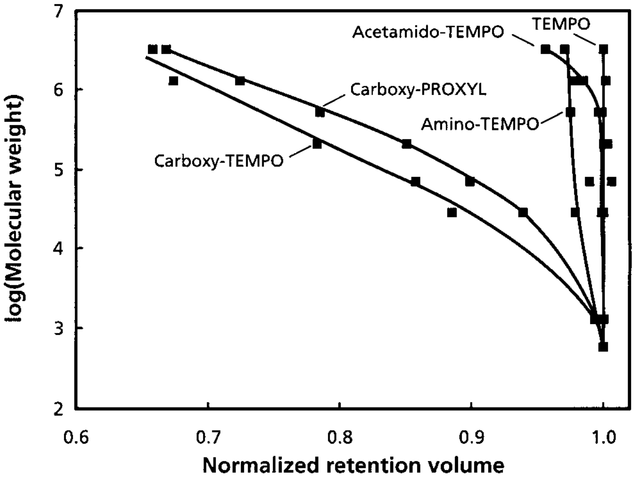

Figure 7. SEC calibration curves of polystyrene for poly(S-*co*-DVB) monoliths (50 mm by 8 mm i.d.) polymerized at 130 °C with PEG:1-decanol ratio of 85:15 using different SFRs with a molar ratio of 1.3 to 1 with respect to BPO.

Effect of SFR Compound. Despite the structural similarities of TEMPO, 3-carboxy-PROXYL, and 4-carboxy-TEMPO, the chemical structure of the SFR used for the mediation of polymerization affected not only the reaction kinetics discussed above but also the pore size distributions of the monoliths. The SEC curve obtained for a column prepared using the polymerization conditions of Figure 4 (PEG:1-decanol ratio 85:15), except for using TEMPO instead of 3-carboxy-PROXYL, is shown in Figure 7. The calibration curve indicates that the monolith contained mainly large pores and had forfeited the size-based separation capability seen for the 3-carboxy-PROXYL mediated polymer. The scanning electron micrograph showing the structural features of monoliths prepared in the presence of TEMPO (Figure 6d) reveals large interconnected globules and large pores between them, whereas much smaller pores are visible within the monolith prepared under identical conditions with 3-carboxy-PROXYL (Figure 6b). Table 1 shows that the surface area values also strongly depend on the SFR chosen. While the monolith prepared with TEMPO had a very low surface area of 1.2 m^2/g, a resin with a high surface area of 72.1 m^2/g was prepared in the polymerization mediated by 3-carboxy-PROXYL.

Since the porosity of the reference polymer synthesized without SFR resembles that of the TEMPO moderated polymer, we wanted to assess whether the unique pore size distribution of the 3-carboxy-PROXYL mediated monoliths originated from the polar and potentially ionic carboxylic functionality of 3-carboxy-PROXYL, rather than the altered polymerization kinetics.[6] As mentioned above, the conversion kinetics for a polymerization carried out in the presence of 4-carboxy-TEMPO (Figure 3) was comparable to that for 3-carboxy-PROXYL. The 4-carboxy-TEMPO moderated polymer also exhibited the highest surface area among the SFRs tested (Table 1). Because surface chemistry apparently plays an important role in the precipitation processes accompanying the formation of a monolith, we investigated other SFRs with polar and charged headgroups (Scheme 1). Monoliths were prepared using 4-acetamido-TEMPO, 4-amino-TEMPO, and 4-trimethylammonio-TEMPO iodide as moderators. The pore size distributions of these monoliths were investigated by SEC, and the calibration curves are shown in Figure 7. The SEC pore size distributions were almost identical for all SFRs tested, except for the monoliths synthesized in the presence of 3-carboxy-PROXYL and 4-carboxy-TEMPO. 4-Trimethylammonio-TEMPO iodide was included in the test since it carries a permanent cationic charge on the quaternary ammonium group, although experimental difficulties associated with limited solubility of 4-trimethylammonio-TEMPO iodide in the polymerization media made the achievable BPO:SFR ratio in the polymerization mixture lower than the intended level of 1:1.3. Nevertheless, SEC measurements indicated no separation capability with respect to molecular weight.

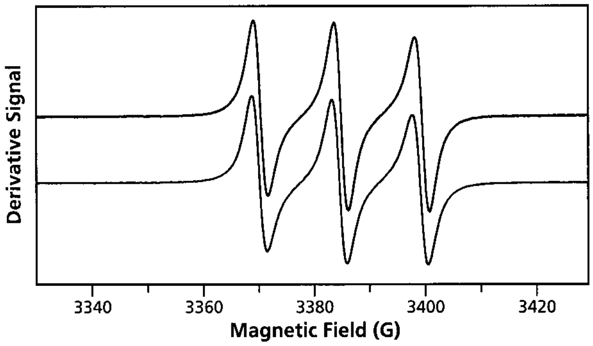

Figure 8. Electron spin resonance spectra of 1.2 mg of 3-carboxy-PROXYL dissolved in 100 mg of 1-decanol and 400 mg of PEG 400 and heated to 130 °C: lower spectrum, 15 min after the heating commenced; upper curve, after heating the sample for 340 min in the cavity.

To ascertain that the carboxy-containing SFRs did not undergo side reactions such as anhydride formation or esterification with the hydroxyl groups containing porogens during polymerizations at a high temperature, 3-carboxy-PROXYL was heated in the typical porogen mixture in the ESR spectrometer cavity at the highest polymerization temperature used in our polymerization experiments (130 °C). Figure 8 shows the ESR spectra obtained after 15 and 340 min heating. Törmäle et al. showed already in 1970 that the line shape and peak-to-peak separation of the ESR spectrum of a free nitroxide radical in solution differ from the ESR spectrum of a nitroxide radical covalently attached to PEG.[18] The spectra corresponding to nitroxide radicals attached to PEG of different molecular weights were asymmetrical due to anisotropy effects. Osterloh et al. have furthermore reported that the ESR spectrum of the biradical corresponding to 3-carboxy-PROXYL anhydride differs in line shape from that of 3-carboxy-PROXYL.[19] Since the spectra in Figure 8 are essentially identical, we can safely conclude that no reactions of 3-carboxy-PROXYL leading to end-labeling of PEG or biradical formation are likely to have occurred in the system.

In-Situ Grafting. The notable advantages of SFR mediated radical polymerizations are an absence of the gel effect[20] and the option of using dormant SFR-capped radicals remaining after the polymerization for controlled grafting of the monolith surface.[6] As mentioned above, our ESR experiments revealed that it was possible to release 3-carboxy-PROXYL radical from porous poly(S-DVB) monoliths synthesized in its presence simply by heating the material in xylene to the temperature required for cleavage of the bond between the polymer terminus and the capping SFR. The release of free radicals during the first 4 min of heating to 399 K, the time period at which the release curve is close to linear, is shown in Figure 9 for TEMPO, 3-carboxy-

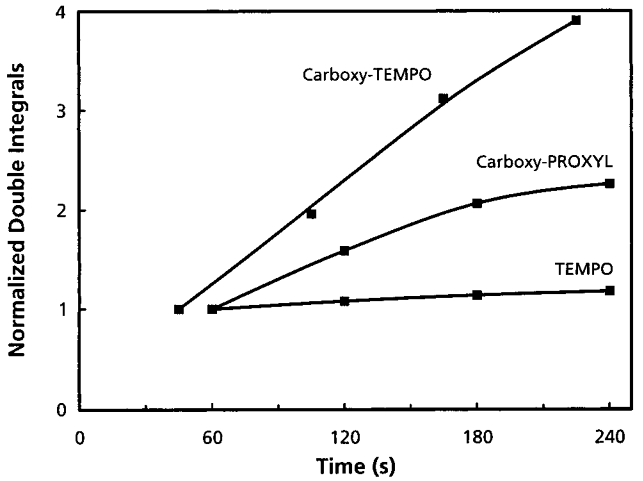

Figure 9. Release of SFR from poly(S-*co*-DVB) monoliths synthesized with 4-carboxy-TEMPO, 3-carboxy-PROXYL, and TEMPO, using BPO:SFR molar ratios of 1:1.3. Prior to heating in the ESR spectrometer, the samples were suspended in xylene, and each measurement was carried out at 399 K. The relative concentration of radicals at each time point is determined from double integration of the ESR spectra, and each value is normalized against the concentration corresponding to the back ground signal of free radical in each polymer sample.

PROXYL, and 4-carboxy-TEMPO. The respective slopes of these curves confirm the results of kinetics experiments, namely, that both the carboxy-functionalized SFRs afford a faster polymerization kinetics than TEMPO.

In our earlier paper concerned with the TEMPO moderated preparation of monoliths,[6] we demonstrated that vinyl monomers could be successfully grafted onto the pore surface. However, the porous properties of the original monoliths did not allow facile flow through the porous structure at acceptable flow rates and backpressures. Therefore, the grafting experiments had to be carried out in batch with crushed polymer. Our new porogenic mixture allows preparation of poly(S-DVB) monoliths with much better permeability. As a result, the monolith does not need to be removed from the mold (column), and the surface grafting can be performed in situ by simply filling the pores with a solution of a monomer and heating to a temperature at which the capped free radicals are liberated.[14] For example, using this approach, the pore surface of poly(S-DVB) monolith prepared in the presence of 3-carboxy-PROXYL was hydrophilized by grafting poly(2-hydroxyethyl methacrylate). Figure 10 shows the effect of this modification on the retention of a homologous series of aromatic compounds using HPLC separation of benzoic acid esters. The retentions of individual homologues in the "plain" monolith increase with increasing length, and hence the hydrophobicity, of the alkyl substituent on the benzoic acid esters injected. This effect is typical of hydrophobic separation media used in the reversed-phase chromatographic mode. In contrast, no retention was observed for the monolith after grafting with poly(HEMA), demonstrating the considerably increased hydrophilicity of the pore surface. The pore volume of the monolith decreased after the grafting by only 11.5%. As a result, there was no significant increase in backpressure. Spontaneous polymerization was not observed even after keeping the HEMA solution used in grafting experiments for 24 h at a temperature of 130 °C in the absence of a monolith.

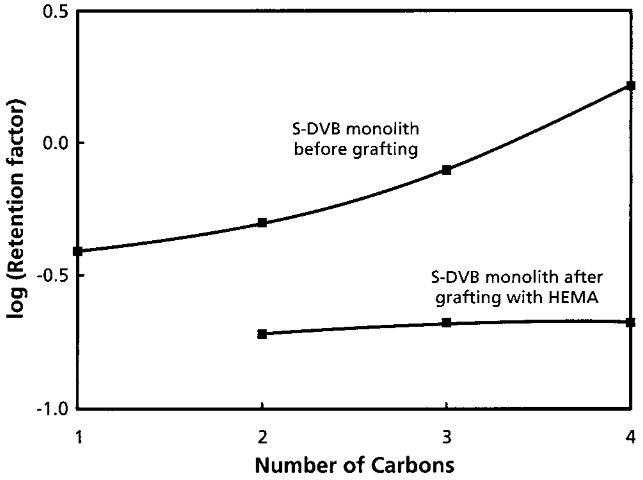

Figure 10. Reversed phase retention behavior of poly(S-*co*-DVB) before and after grafting with poly-HEMA, as determined by the retention increase vs the number of carbons in the side chains of a homologous series of aromatic benzoic acid esters. The poly(S-*co*-DVB) monolith was synthesized from a polymerization solution containing 4 parts by weight of monomers and 6 parts of porogen (PEG:1-decanol 4:1) and subsequently polymerized at 130 °C using a BPO to 3-carboxy-PROXYL ratio of 1:1.1.

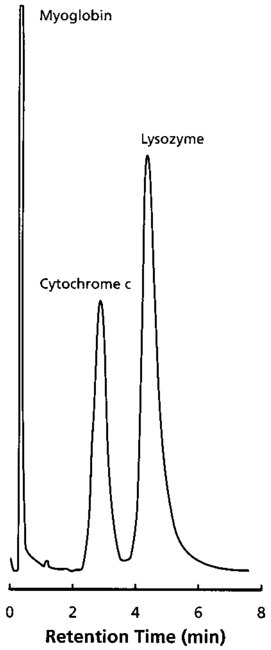

Figure 11. Cation-exchange separation of 20 μL of a protein mixture (0.5 mg/mL with respect to each protein) using a poly(S-*co*-DVB) monolith grafted with 3-sulfopropyl methacrylate in DMSO. Linear gradient ranging from 20 to 100% of 0.25 M NaCl in 20 mM phosphate buffer pH 7, at a flow rate of 1.0 mL/min.

A similar procedure was also used for grafting 3-sulfopropyl methacrylate. This reaction converted the originally hydrophobic poly(S-DVB) monolith into a highly polar strong cation exchanger. The monolith was prepared in a smaller 50 by 2.6 mm i.d. column using 3-carboxy-PROXYL mediated polymerization, grafted with 3-sulfopropyl methacrylate, and tested in cation-exchange chromatographic mode. Figure 11 shows the separation of three proteins—myoglobin, cytochrome *c*, and lysozyme—using a linear NaCl gradient as the eluent. The peaks are sharp, indicative of a surface covered with a homogeneous layer of charged functionalities not leaving behind any hydrophobic patches. The amount of sulfopropyl groups within the grafted monolith can be evaluated from their ion-exchange capacity. The material was first loaded with Mg^{2+} ions that were then displaced with Ca^{2+} ions, and the amount of released Mg^{2+} ions was determined by atomic absorption spectrometry. Using this procedure, an ion-exchange capacity of 37 μmol of Mg^{2+}/g of polymer was found for one of our grafted monoliths.

Conclusions

The method developed in this study allowed for the first time the preparation of "molded" porous monoliths in the presence of *stable free radicals* with high flow permeability suitable as chromatographic separation media. The combination of 3-carboxy-PROXYL or 4-carboxy-TEMPO mediators and the newly developed porogenic system comprising poly(ethylene glycol) and 1-decanol afforded poly(S-DVB) monoliths with porous properties different from those found in monoliths produced by polymerizations involving other SFRs. Our present monoliths are characterized by both large surface areas and large through-pores. The broad pore size distribution of these monoliths is also suitable for their use in separation of polymers in SEC mode. This application will be further explored using SEC columns with conventional dimensions. The suitable porous properties also enable grafting of the pores with functional vinyl monomers in situ to easily control the pore surface chemistry.

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