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Thermoresponsive Oligo(ethylene glycol)-Based Polymer Brushes on Polymer Monoliths for All-Aqueous Chromatography

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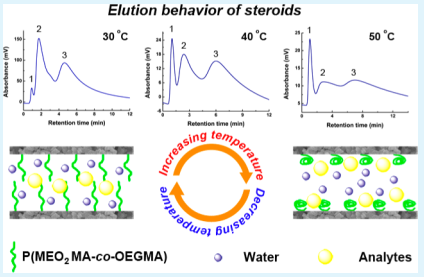
Supporting Information

ABSTRACT: Porous polymer monoliths onto which were grafted a thermoresponsive copolymer, poly(2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA)-*co*-oligo(ethylene glycol) methacrylate (OEGMA)), were synthesized by the two-step atom transfer radical polymerization (ATRP) method. The copolymer-grafted monoliths were characterized by elemental analysis, scanning electron microscopy, and mercury intrusion porosimetry. They were further used as the thermoresponsive stationary phase for all-aqueous high-performance liquid chromatography (HPLC). The chromatograms of three steroids demonstrated that the chain length of the grafted copolymer, which was regulated by varying the grafting time, could affect the separation by providing different amounts of hydrophobic interaction sites with analytes. Additionally, the elution profiles of steroids on the stationary phase could also be tuned by the comonomer composition. The results showed that the porous polymer monoliths enabled separation of the test mixture in pure aqueous mobile phase under isocratic conditions. Furthermore, the proposed method provides a simple and promising tool in the design and construction of responsive surfaces for chromatography applications.



KEYWORDS: *poly(2-(2-methoxyethoxy)ethyl methacrylate-co-oligo(ethylene glycol) methacrylate) brushes, two-step atom transfer radical polymerization, thermoresponsive monolith, separation, hydrophobic interaction*

INTRODUCTION

Responsive surfaces, usually prepared by modification of responsive polymer brushes on solid substrates, are of current interest in a number of research areas, including biosensing, cell culture, and drug delivery.[1−4] One of the most interesting applications is responsive stationary phases.[5,6] Especially, thermoresponsive stationary phases, emerging as a new class of chromatography supports for separation of bioanalytes, have been drawing a considerable amount of attention.[7−10] The main advantage of thermoresponsive chromatography is that the separation can be achieved by only changing the column temperature without using an organic solvent as the mobile phase. Thus, it contributes to maintaining the biological activity of analytes and reducing the environmental burden. To date, most thermoresponsive stationary phases for all-aqueous chromatography have been produced from poly(*N*-isopropylacrylamide) (PNIPAM),[8,11] which exhibits a lower critical solution temperature (LCST) of 32 °C in water.[12] Thus, the hydrophobic property of the PNIPAM-grafted surface could be easily altered when the temperature is changed across the LCST. Recently, polymers with oligo(ethylene glycol) side chains have been attracting a lot of attention as a new family of thermoresponsive polymers.[13] Among them, copolymers of 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA) and oligo(ethylene glycol) methacrylate (OEGMA) were reported to exhibit LCST values that can be tuned in the range of 26−90 °C by varying the co-monomer composition.[14] In addition, their LCST values were found to be less affected by factors such as ionic strength, concentration of the copolymer in water, and chain length.[15] Therefore, the P(MEO_2MA-*co*-OEGMA) copolymer appears as a reliable choice instead of conventional PNIPAM for chromatography applications. However, oligo(ethylene glycol)-based thermoresponsive stationary phases have been constructed only on silica monoliths.[16]

Porous polymer monoliths were developed in the early 1990s and have been occupying an important position in separation science because of the simple fabrication, improved mass transfer properties, and good tolerance to extreme pH.[17−19] However, research on thermoresponsive chromatography based on polymer monoliths has remained scarce. Thus, introducing novel thermoresponsive polymer brushes onto polymer monolith surfaces, combining the advantages of two kinds of materials in chromatography applications, is necessary and desirable. Among the several techniques to prepare polymer monoliths, atom transfer radical polymerization (ATRP), which is the most versatile method of living free-radical polymerization,[20,21] offers great advantages such as allowing reaction under mild conditions and control of the grafting polymer length.[22−25] Therefore, the two-step ATRP method could

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provide a simple and effective approach for preparing and grafting monoliths to generate responsive stationary phases.

In this study, P(MEO_2MA-*co*-OEGMA)-grafted porous polymer monoliths were prepared via the two-step ATRP method and applied as the novel stationary phase for thermoresponsive chromatography. P(MEO_2MA-*co*-OEGMA) brushes with different chain lengths for various interaction sites were obtained by changing the grafting time. Moreover, the comonomer composition was altered to modulate the hydrophobicity of P(MEO_2MA-*co*-OEGMA). Characterizations of the responsive copolymer-grafted polymer monoliths were well-performed. Furthermore, the separation abilities of the prepared columns were evaluated using three steroids in high-performance liquid chromatography (HPLC).

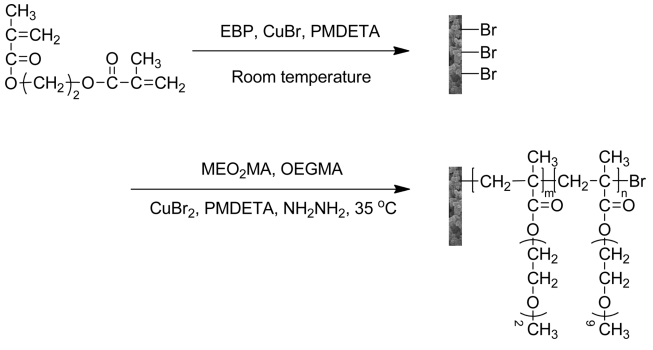
EXPERIMENTAL SECTION

Materials. Ethylene glycol dimethacrylate (EDMA) was freshly distilled under vacuum prior to use. Cuprous bromide (CuBr) was washed with acetic acid and methanol and vacuum-dried before use. 2-(Dimethylamino)ethyl methacrylate (DMAEMA), ethyl 2-bromopropionate (EBP), 1,1,4,7,7-pentamethyldiethylenetriamine (PMDETA), cupric bromide ($CuBr_2$), MEO_2MA, OEGMA (M_n = 475 g/mol), hydrocortisone, testosterone, medroxyprogesterone acetate, and other chemicals were commercially available and used directly without purification. Milli-Q water prepared by an ultrapure water purification system (Millipore, Billerica, MA) was used in this study.

Preparation of Porous Monoliths. The porous polymer monoliths were synthesized by ATRP. The detailed preparation process was similar to that reported by our lab[26] and is described as follows: A mixture of EDMA (0.5 mL, 0.26 mmol), EBP (1.9 μL, 0.015 mmol), cuprous bromide (6.5 mg, 0.045 mmol), methanol (0.5 mL), and hexane (0.5 mL) was placed in a dry sample vial, homogenized by ultrasonic waves, and then deoxygenized by purging with Ar for 10 min. Subsequently, PMDETA (9.2 μL, 0.045 mmol) was quickly added to the mixture, and the solution was injected into a 50 mm × 4.6 mm I.D. column. With both ends sealed, the column was placed at room temperature to react for 12 h and then was connected to the HPLC system. The residual compounds that may remain in the polymer monolith were washed out by pumping 100 mL of methanol and 50 mL of water successively through the column at a flow rate of 0.20 mL/min.

Grafting of Porous Monoliths. The grafting of P(MEO_2MA-*co*-OEGMA) on prepared monoliths via surface-initiated activators regenerated by electron transfer atom transfer radical polymerization (ARGET ATRP) was performed as displayed in Scheme 1. The detailed polymerization procedure was as follows: $CuBr_2$ (34 mg, 0.15 mmol), PMDETA (32 μL, 0.15 mmol), MEO_2MA (0.76 mL, 4.11 mmol), and OEGMA (0.14 mL, 0.32 mmol) were dissolved in 20 mL of water. The mixture formed a uniform blue solution under ultrasonic waves. Next, hydrazine (37 μL, 0.6 mmol) was added to the above solution, and the resulting mixture was sonicated for 10 s. The color of the mixture was found to turn reseda at the same time. Subsequently, the reaction was carried out at 35 °C by pumping the polymerization solution through the polymer monolith at a flow rate of 0.05 mL/min. After 4 h of grafting polymerization, the monolith was washed with 100 mL of water to wash out all of the soluble residues remaining in the monolithic column.

Scheme 1. Route for Preparing the P(MEO_2MA-*co*-OEGMA)-Grafted Polymer Monoliths by the Two-Step ATRP Method



Characterization of the Prepared Monoliths. The prepared porous monoliths (both nongrafted and grafted) were removed from the columns and subjected to elemental analysis using a Flash EA 1112 elemental analyzer. The amount of the bromic group, which served as the surface initiator for grafting polymerization, was calculated using the equation

$$\text{ATRP initiator} = \frac{\%\text{Br} \times 10^6}{\%\text{Br}^{\text{calcd}}\left[1 - \frac{\%\text{Br}}{\%\text{Br}^{\text{calcd}}}\right]MS}$$

where %Br is the percent bromine as determined by elemental analysis, $\%\text{Br}^{\text{calcd}}$ is the calculated weight percent of bromine in the initiator unit, M is the formula weight of the initiator unit (g/mol), and S is the specific area of the prepared monolith.

The amount of grafted copolymer on the polymer monolith surface was calculated using the equation

$$\text{grafted copolymer} = \frac{\%\text{C}_\text{p}}{\%\text{C}_\text{p}^{\text{calcd}}\left[1 - \frac{\%\text{C}_\text{p}}{\%\text{C}_\text{p}^{\text{calcd}}} - \frac{\%\text{C}_\text{i}}{\%\text{C}_\text{i}^{\text{calcd}}}\right]S}$$

where %C is the percent carbon increase over that of the original monolith as determined by elemental analysis and $\%\text{C}^{\text{calcd}}$ is the calculated weight percent of carbon in the initiator or copolymer monomer. The subscript i denotes the initiator ($\%\text{C}_\text{i}$ equals zero because the original monolith itself served as the macroinitiator) and the subscript p denotes the copolymer.

The morphologies of the prepared monoliths were characterized using scanning electron microscopy (SEM) on a model S-4300 scanning electron microscope (Hitachi, Japan). Mercury intrusion porosimetry was performed to determine the pore size distribution of the prepared monoliths on an Autopore III 9220 mercury intrusion porosimeter (Micromeritics, USA).

Temperature-Modulated Elution of Steroids. The P(MEO_2MA-*co*-OEGMA)-grafted polymer monoliths were connected to an HPLC system (LC-20A, Shimadzu, Japan) with a UV−vis detector (SPD-20A) for chromatographic evaluation. Hydrocortisone, testosterone, and medroxyprogesterone acetate were selected as model analytes at concentrations of 0.02, 0.42, and 0.75 mg/mL, respectively. Table 1 presents the molecular weight and log*P* values of the steroids.

Table 1. Properties of Model Steroids

analyte	mol wt (g/mol)	log*P*[a]
hydrocortisone	360.49	1.96
testosterone	288.42	2.44
medroxyprogesterone acetate	384.51	3.31

[a]Partition coefficient in the *n*-octanol/water system.

Milli-Q water was pumped through the monolith column as the mobile phase, and the elution behavior of the analytes was recorded with a flow rate of 1.0 mL/min at different temperatures. The detection wavelength was set at 254 nm. Van't Hoff plots were built to investigate the retention behavior of the analytes on the thermoresponsive monolithic columns. The value of the retention factor k' was calculated as

$$k' = (t_\text{R} - t_0)/t_0$$

where t_R is the retention time of the model steroid at a specific temperature and t_0 is the retention time of potassium nitrate.

Table 2. Characterization of P(MEO_2MA-*co*-OEGMA)-Grafted Monoliths

monolith[a]	grafting time (h)	elemental composition (%)[b] C	elemental composition (%)[b] Br	amount of initiator (μmol/m^2)[c]	grafted polymer (mg/m^2)[d]	MEO_2MA/OEGMA molar ratio in the feed
C0-0	0	58.40 ± 0.17	1.90 ± 0.15	119		
C3-15	3	59.70 ± 0.09	<0.30		11.56	85/15
C4-15	4	59.90 ± 0.01	<0.30		13.39	85/15
C8-15	8	63.46 ± 0.01	<0.30		48.24	85/15
C4-10	4	59.80 ± 0.12	<0.30		12.46	90/10
C4-20	4	59.90 ± 0.03	<0.30		13.37	80/20

[a]Thermoresponsive-copolymer-grafted poly(EDMA) monoliths are named as C*x*-*y*, where *x* represents the grafting time and *y* represents the feed mole fraction of OEGMA in the copolymer. [b]Determined by elemental analysis (n = 2). [c]Estimated from the bromine composition. [d]Estimated from the carbon composition.

RESULTS AND DISCUSSION

Preparation and Grafting of Porous Monoliths. The ATRP method was adopted in this work to fabricate the poly(EDMA) monoliths at room temperature. The preparation procedure was more convenient in operation compared with conventional radical polymerization.[27−29] The prepared monolith, which contained bromic groups on its surface, was employed as both the substrate for chromatographic applications and the ATRP macroinitiator for subsequent grafting. In the aspect of grafting polymer chains on solid substrates, surface-initiated ATRP is a most extensively employed method that allows precise control over the length and composition of polymer chains. However, typical ATRP should be carried out under strictly anaerobic conditions because oxygen can induce deactivation of the metal catalyst and produce unreactive peroxy radicals,[30] leading to termination of the polymerization. To avoid a complicated deoxygenation procedure such as freeze−pump−thaw cycles,[31] ARGET ATRP, which can tolerate a small amount of air without sacrificing the advantages of normal ATRP,[32] was employed to graft P(MEO_2MA-*co*-OEGMA) copolymer on the polymer monoliths in this study. Hydrazine was used for reduction of Cu^{II} to Cu^{I}. It could be observed that the color changed from blue to reseda after hydrazine was added, indicating the formation of Cu^{I}.

Previous literature reports have demonstrated that interactions between polymer chains and analytes can be controlled by optimization of the amount of polymer grafted on monoliths or beads.[33−35] However, the amount of grafted polymer, which is determined by both the grafting density and the chain length of the polymer,[25] is difficult to tune by changing the former factor in the present two-step ATRP approach because the polymerization reaction would become too fast with a high initiator concentration, leading to difficulty in operation. Thus, altering the copolymer chain length by changing grafting time was adopted to optimize the experimental conditions, and monolithic columns with 3, 4, and 8 h of grafting time were prepared. Moreover, since the LCST of P(MEO_2MA-*co*-OEGMA) could be influenced by the comonomer composition, monolithic columns with different OEGMA compositions were also prepared.

Characterization of the Thermoresponsive-Copolymer-Grafted Monolithic Columns. For measuring the amount of surface initiator and grafted copolymer on monolithic surfaces, the prepared monoliths were characterized by elemental analysis. The elemental compositions of carbon and bromine are displayed in Table 2. The columns are abbreviated as C*x*-*y*, where *x* and *y* represent the grafting time and mole fraction of OEGMA in the feed, respectively. In those columns grafted with P(MEO_2MA-*co*-OEGMA), the carbon content increased relative to that in the nongrafted column. After grafting polymerization, a lower bromine composition (less than 0.3%) was observed as a result of the increasing content of other elements that P(MEO_2MA-*co*-OEGMA) contains, although the bromine remained on the polymer terminal of the grafted polymer after the grafting polymerization was completed.[36] These results indicated that the thermoresponsive copolymers were successfully grafted on the monolith surfaces. It should be noted that the calculated amount of the grafted copolymer on the prepared polymer monolith was higher than those on silica monoliths and silica beads. This was the case because of the relatively smaller specific surface area (2.0 m^2/g) attributed to much larger pores and the low numbers of micropores and mesopores, which were comparable to those reported in the literature for polymer monoliths.[37,38] Furthermore, comparisons among C3-15, C4-15, and C8-15 indicated that the amount of the grafted copolymers increased with increasing grafting time.

Since the grafted P(MEO_2MA-*co*-OEGMA) brushes could not be separated from the poly(EDMA) monolith surface, free P(MEO_2MA-*co*-OEGMA) copolymers were prepared in solution via ARGET ATRP for further investigation of the properties of their corresponding grafted ones. The synthetic procedure was the same as that reported in the literature[39−41] and is described in the Supporting Information. The characteristics of the thermoresponsive copolymers are summarized in Table S1 in the Supporting Information. The prepared free copolymers are abbreviated as P*x*-*y*, where *x* denotes the preparation time and *y* denotes the feed mole fraction of OEGMA. P3-15, P4-15, and P8-15 with the prolonged preparation times were found to have increasing molecular weight. It was also observed that the LCST values increased as the feed mole fraction of the OEGMA monomer increased because of the higher content of the more hydrophilic monomer.[42] The phase transition profiles of the copolymers are shown in Figure S3 in the Supporting Information.

SEM observations were performed to compare the morphology of the grafted monolithic materials with that of their nongrafted counterparts (Figure 1). A larger number of through-pores was found in the nongrafted monolith. The result was also confirmed by the pore size distribution curves of the prepared polymer monolith. As displayed in Figure 2, mercury intrusion porosimetry showed a pore size centered around 4.5 μm for the original monolith, while that of the grafted monolith was 2.5 μm. It is worth mentioning that these large through-pores would afford good hydrodynamic proper-

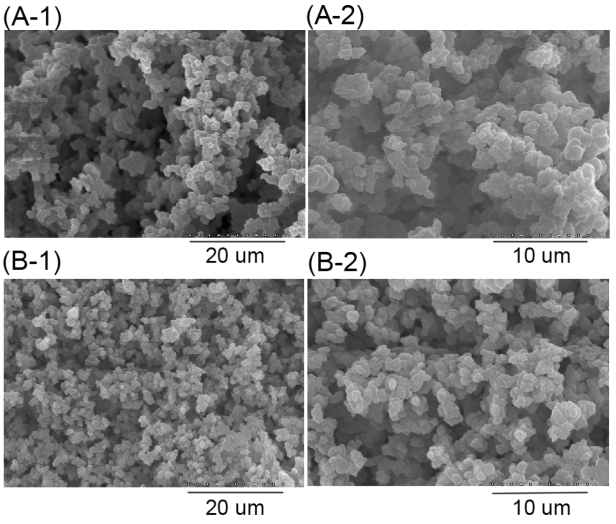


Figure 1. SEM images of the prepared polymer monolithic materials: (A) nongrafted polymer monolith; (B) P(MEO_2MA-*co*-OEGMA)-grafted polymer monolith. The images (A-2) and (B-2) are high-magnification images of (A-1) and (B-1), respectively.

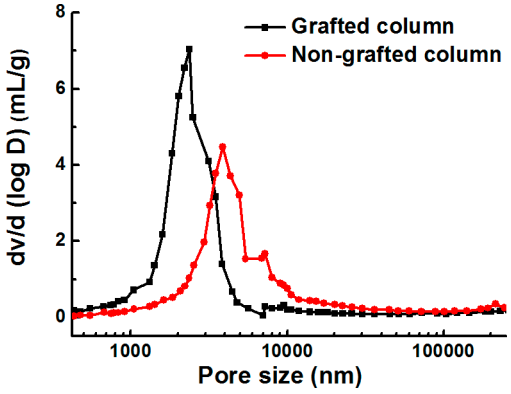


Figure 2. Pore size distribution curves of the original and P(MEO_2MA-*co*-OEGMA)-grafted monoliths as determined by mercury intrusion porosimetry.

ties and allow for high flow rates in liquid chromatography. Both results undoubtedly demonstrated the successful grafting of the P(MEO_2MA-*co*-OEGMA).

Elution Behavior of Model Steroids on Monolithic Columns with Different Chain Lengths of the Copolymers. Figure 3 shows the retention times of the model steroids on C3-15, C4-15, and C8-15. On all of the columns, the retention times increased in the order hydrocortisone (1) < testosterone (2) < medroxyprogesterone acetate (3), in agreement with the hydrophobicities of the analytes. The result indicated that the separation of the analytes was mainly driven by the hydrophobic−hydrophobic interactions between the three steroids and the stationary phase. Additionally, with increasing chain length of P(MEO_2MA-*co*-OEGMA), the retention times for the analytes became longer as a result of the enhanced hydrophobic interaction. The elution profiles of an aqueous mixture of the analytes on a nongrafted column and on columns with different grafting times are displayed in Figure 4. We found that when the nongrafted monolith was used, medroxyprogesterone acetate was not eluted from the stationary phase. This was attributed to the fact that the hydrophobic interaction between medroxyprogesterone acetate

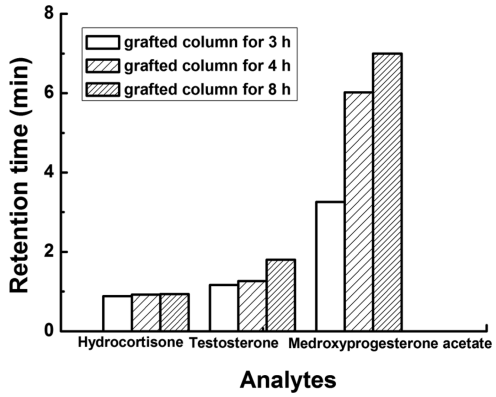


Figure 3. Effect of grafting time on the retention time of the model analytes at 40 °C.

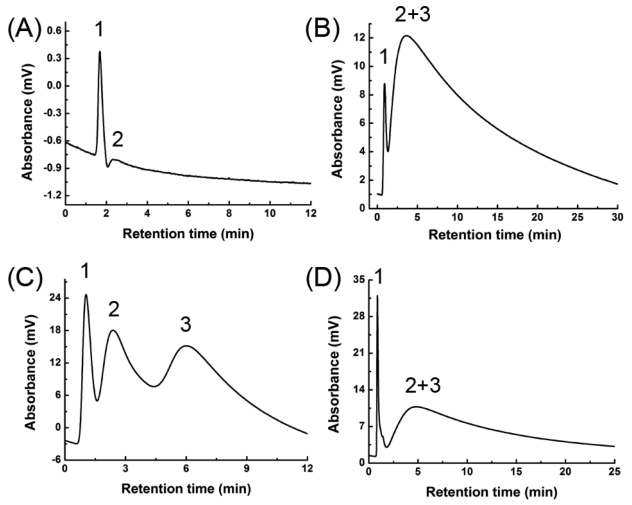


Figure 4. Elution profiles of aqueous mixtures of the steroids at 40 °C on (A) C0, (B) C3-15, (C) C4-15, and (D) C8-15. Peaks 1, 2, and 3 represent hydrocortisone, testosterone, and medroxyprogesterone acetate, respectively.

and the poly(EDMA) monolith was too strong to allow elution using pure water as the mobile phase. Testosterone and medroxyprogesterone acetate eluted in a single peak on C3-15, as the short chain length of P(MEO_2MA-*co*-OEGMA) produced insufficient interaction sites to separate the steroids. In contrast, unresolved peaks were also observed using C8-15 with the longest chain length as a result of partitioning of the steroids into the thick grafted copolymer brush layers.[43] The three analytes can be separated well on C4-15. Thus, the monolith with 4 h of grafting time was finally selected for all further chromatographic evaluations. Moreover, it should be mentioned that the chromatographic performance of the newly prepared polymer monolith grafted with P(MEO_2MA-*co*-OEGMA) could be further improved by increasing the separation column length and altering the grafting conditions, as displayed in Figure S4 in the Supporting Information for actual analysis in the future.

Temperature-Modulated Elution of Steroids. To investigate the surface hydrophobicity of the prepared monolithic columns, the temperature-modulated elution profiles of the analytes were studied. Figure 5A−C presents the chromatograms of the analytes at different temperatures on C4-10, C4-15, and C4-20. The different elution profiles can be explained by the alterations of the hydrophobicity of the

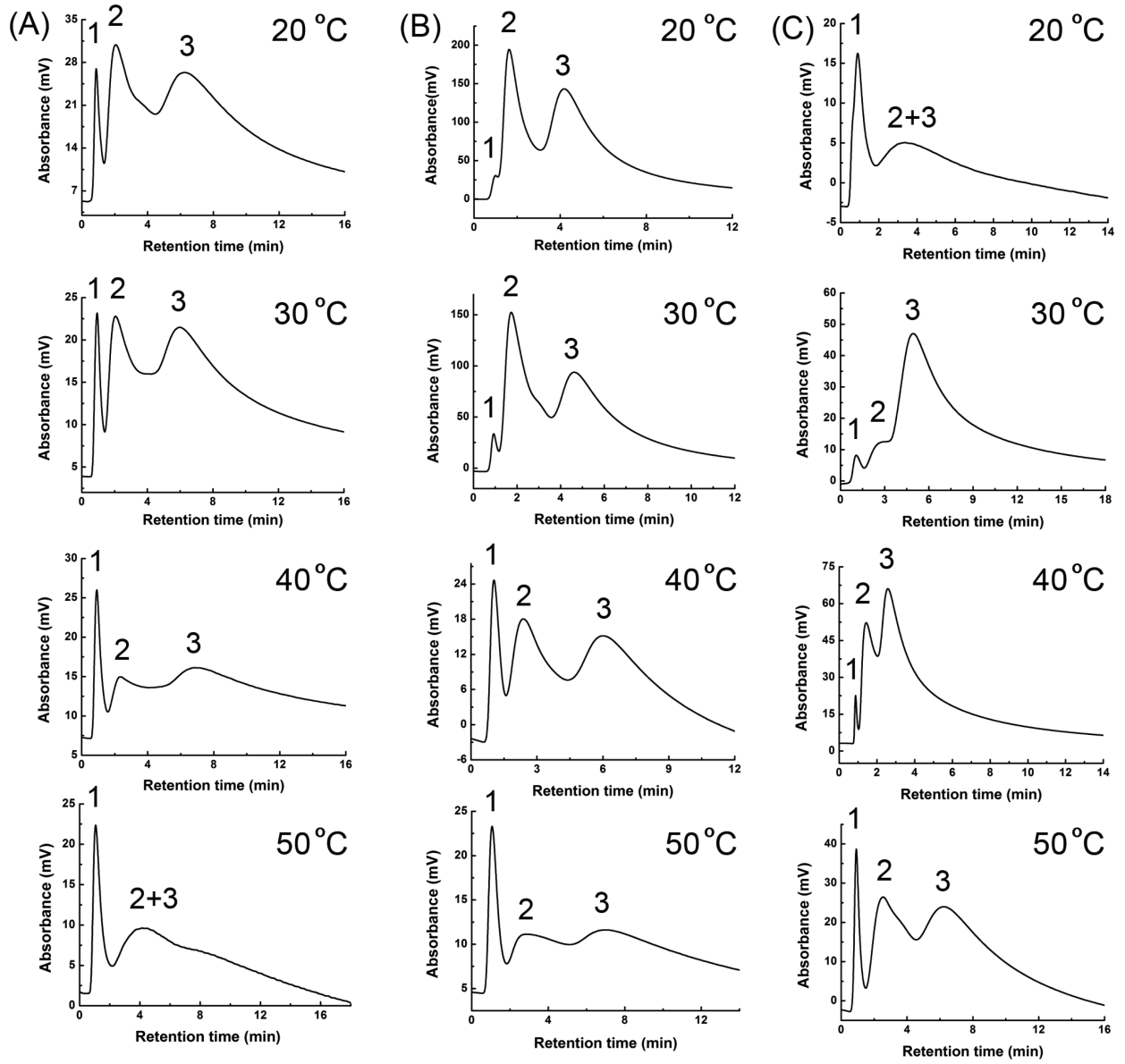


Figure 5. Elution profiles of an aqueous mixture of steroids at different temperatures on (A) C4-10, (B) C4-15, and (C) C4-20. Peaks 1, 2, and 3 represent hydrocortisone, testosterone, and medroxyprogesterone acetate, respectively.

thermoresponsive stationary phase. Since P(MEO_2MA-*co*-OEGMA) exhibits an aqueous LCST, its thermoresponsive behavior results from the balance between hydrophilic P(OEGMA) and hydrophobic P(MEO_2MA) in the structure.[44] With increasing temperature, the balance between favorable copolymer−water interactions and unfavorable hydrophobic interactions is disrupted, resulting in high hydrophobicity of the P(MEO_2MA-*co*-OEGMA)-grafted monolith surfaces. Meanwhile, along with the variation of the hydrophobicity of the grafted monolith, the conformation of the P(MEO_2MA-*co*-OEGMA) brushes changes gradually from extended structures into coiled ones as the temperature increases. Therefore, when the grafted monolith was employed to separate hydrophobic steroids, the separation process can be described as follows: decreasing the temperature extends the P(MEO_2MA-*co*-OEGMA), allowing the analytes to be eluted from the stationary phase, and increasing the temperature induces collapse of the copolymer brushes with high hydrophobicity, resulting in strong retention of the analytes on the stationary phase (Figure 6). Figure 5 shows that hydrocortisone and testosterone cannot be effectively separated at low temperature on C4-15 and C4-20. The best separation of the three steroids on C4-10, C4-15, and C4-20 could be achieved at 30, 40, and 50 °C, respectively. However, further increasing the temperature resulted in lower separation resolution of testosterone and medroxyprogesterone acetate. To investigate this un-

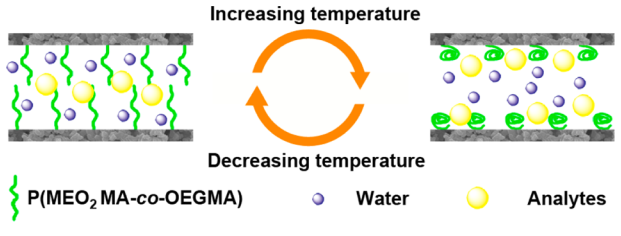


Figure 6. Separation mechanism of the steroid mixture on the P(MEO_2MA-*co*-OEGMA)-grafted thermoresponsive stationary phase.

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expected result in detail, temperature-dependent peak width changes of the three steroids were studied on C4-15 as an example. As depicted in Figure 7, we found that the peak

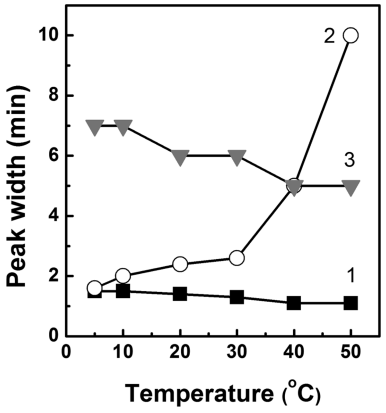

Figure 7. Temperature-dependent peak width changes of the three steroids on C4-15. Peaks: 1, hydrocortisone; 2, testosterone; 3, medroxyprogesterone acetate.

widths of hydrocortisone and medroxyprogesterone acetate decreased with increasing column temperature. Meanwhile, the elution peak of testosterone became broader in shape as the temperature increased. In thermoresponsive chromatography, the peak width of a steroid is affected by multiple factors.[45] In general, the enhanced hydrophobic interactions at elevated temperature lead to peak broadening. But an opposite effect is that shrinking of the P(MEO_2MA-*co*-OEGMA) brushes with increasing temperature prevented penetration of the steroid molecules into the copolymer brushes, resulting in a decrease of the peak width. Additionally, the solubility of the steroids in the mobile phase increased as the temperature increased, also bringing about a narrower peak. Therefore, the elution profiles of the test steroids depended on which factor was dominant. In this system, penetration into the polymer brushes and solubility had larger effects on the peak width for hydrocortisone and medroxyprogesterone acetate, while the enhanced hydrophobic interactions were observed to be more important for testosterone. The result further explained why testosterone and medroxyprogesterone acetate could not be separated well at high temperature (Figure 5A,B at 50 °C).

Figure 8A–C shows the changes of the retention times for the analytes at different temperatures on the three prepared columns. The retention times of the analytes on C4-10, C4-15, and C4-20 became longer when the column temperature was increased in the range of 10–30, 20–40, and 40–50 °C, respectively. This was due to the increasing hydrophobic interactions between the three columns and the analytes in these temperature regions. However, no obvious increases and even slight decreases in the retention times were observed with further increases in the temperature, indicating that the hydrophobicity of the monolith surface no longer changed. Interestingly, for more hydrophobic testosterone and medroxyprogesterone acetate, larger changes in the retention times on the three columns were observed in the range of 20–30, 30–40, and 40–50 °C, respectively, indicating that conformational changes occurred in these temperature regions. On the basis of these results, the LCST values of the copolymers grafted on the C4-10, C4-15, and C4-20 columns were speculated to be in these temperature ranges. The free copolymers P4-10, P4-15, and P4-20, which have the same feed compositions as their corresponding grafted P(MEO_2MA-*co*-OEGMA) copolymers, were found to afford the LCST values of 25.7, 36.8, and 44.1 °C, respectively (Table S1 in the Supporting Information). The results were in good accordance with previously reported values[14] and could further confirm our estimation. We also observed that the retention times of testosterone and medroxyprogesterone acetate on C4-10 were longer than on C4-15 and C4-20 (Figure 8), which can be explained by the relatively larger amount of hydrophobic MEO_2MA feed composition in the grafted copolymer on C4-10.

Figure 9A–C presents van't Hoff plots for the model steroids on these three columns. The van't Hoff plots were built by plotting the ln k' values of the analytes against the reciprocal of the column temperature ($1/T$). It was found that the retention factors of the analytes increased with increasing column temperature. It is worth mentioning that although van't Hoff plots are typically linear in conventional reversed-phase chromatography,[10] turning points can be observed in the van't Hoff plots of the analytes on all of these columns around their LCST values, demonstrating the alterations of the hydrophobicity of the stationary phases.

Repeatability of the Thermoresponsive Monolithic Columns. The repeatability of the P(MEO_2MA-*co*-OEGMA)-grafted polymer monoliths was evaluated by the relative

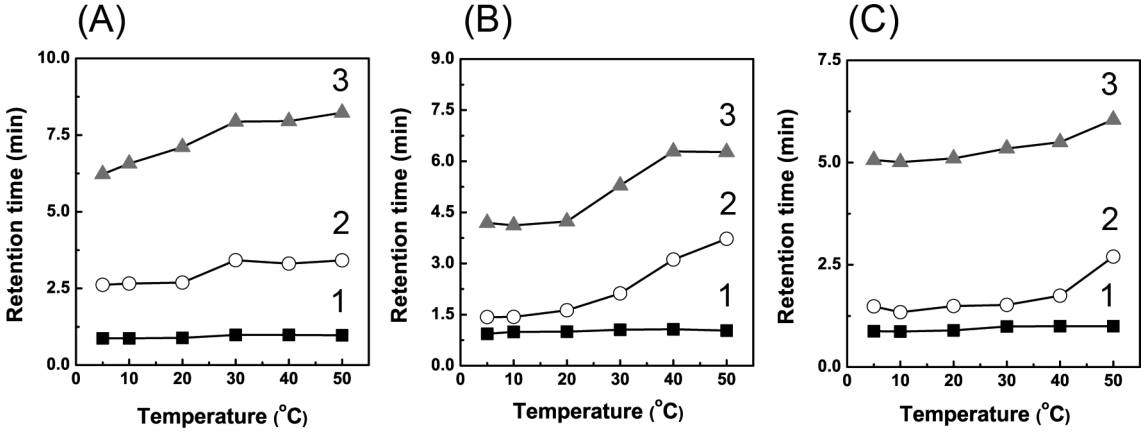

Figure 8. Temperature-dependent retention time changes of model steroids on (A) C4-10, (B) C4-15 and (C) C4-20. Peaks: 1, hydrocortisone; 2, testosterone; 3, medroxyprogesterone acetate.

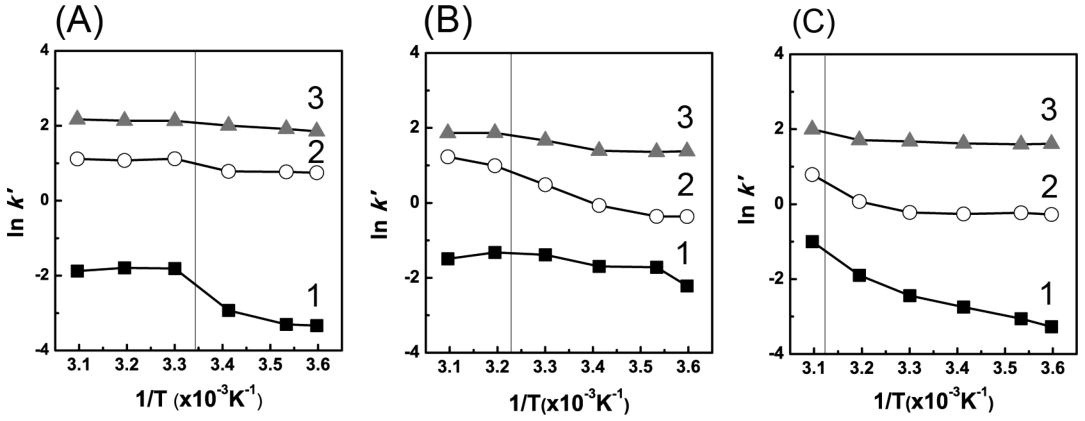

Figure 9. Van't Hoff plots of model steroids on (A) C4-10, (B) C4-15, and (C) C4-20. Peaks: 1, hydrocortisone; 2, testosterone; 3, medroxyprogesterone acetate. The black vertical lines indicate the LCST values of the prepared free P(MEO_2MA-*co*-OEGMA) copolymers.

standard deviations (RSD) of the retention times of the three analytes. Three monolithic columns prepared under the same grafting conditions were used to measure the column-to-column repeatability of the three steroids. The obtained RSD values (n = 3) for hydrocortisone, testosterone, and medroxyprogesterone acetate were 1.7%, 1.2%, and 1.9%, respectively. The run-to-run repeatability was assessed by repeated separation of the mixture under constant conditions. The RSD values (n = 6) of the test analytes were 0.13%, 0.09%, and 0.24%, respectively. The satisfactory repeatability of the prepared monoliths indicated that the present method for preparing polymer monoliths grafted with copolymer brushes is universally applicable.

CONCLUSION

Porous polymer monoliths grafted with thermoresponsive P(MEO_2MA-*co*-OEGMA) brushes were successfully prepared by the two-step ATRP method. The first ATRP step, which was employed to prepare the monolith, was conducted at room temperature for operational convenience. The second ARGET ATRP step allowed the chain length of the grafted polymer to be controlled simply by altering the grafting time. The resulting monolithic materials were applied as stationary phases for thermoresponsive chromatography. The hydrophobicity of the P(MEO_2MA-*co*-OEGMA)-grafted polymer monolith could be modulated by temperature, which led to the separation of the test steroid mixtures in an all-aqueous mobile phase. In addition, the retention times of the three test steroids could be adjusted by varying the copolymer chain length, indicating that ATRP method is an effective tool for regulating the hydrophobicity property of the stationary phase. Meanwhile, the hydrophobic interactions between the steroids and stationary phase also could be tuned by altering the comonomer composition. Furthermore, P(MEO_2MA-*co*-OEGMA)-grafted polymer monoliths provide a promising alternative to existing thermoresponsive polymer monoliths for chromatography and expand the application of polymer monoliths in chromatographic mode.

ASSOCIATED CONTENT

Supporting Information

Experimental procedure for preparing copolymer P(MEO_2MA-*co*-OEGMA) and phase transition profiles of prepared P(MEO_2MA-*co*-OEGMA). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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