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# Preparation and characterization of neutral poly(ethylene glycol) methacrylate-based monolith for normal phase liquid chromatography

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ABSTRACT

A novel porous poly(ethylene glycol) methacrylate-based monolithic column for normal phase liquid chromatography was prepared by thermally initiated polymerization of poly(ethylene glycol) methyl ether methacrylate (PEGMEMA) and ethylene dimethacrylate (EDMA) in the presence of selected porogens. The monolith was macroscopically homogeneous, had low flow resistance, and did not swell or shrink significantly in solvents of different polarities. Inverse size-exclusion data indicate that the monolith had a total porosity of 79.2%, including an external porosity of 69.3% and an internal porosity of 9.9%. Due to its mild polarity (hydrophilicity), the PEG-functionalized monolith could perform traditional normal phase chromatography using non-polar solvents The van Deemter plot demonstrated that the column efficiency of 33,600–34,320 theoretical plates/m could be achieved at a linear flow velocity of 0.9–1.5 mm/s. The dual retention capability (both weak hydrophilic and hydrophobic interactions) investigated in this paper explains well why the PEG-functionalized monolith could operate in various chromatographic modes.

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## 1. Introduction

Among the more recent developments in column technology for liquid chromatography (LC) is the monolithic bed [1–5]. In concept, monolithic columns should reduce the analysis time through low column backpressure, allowing high mobile phase flow rates and fast transfer kinetics. Organic polymers [1–3] and silica [4,5] represent two major monolithic support materials. Organic polymer-based monoliths are particularly attractive due to the wide variety of available chemistries and techniques that can be utilized in their preparation [6]. Polymer monolithic stationary phases for reversed-phase chromatography (RPC) are mainly based on polymethacrylates with alkyl functional groups (from butyl to stearyl methacrylate) [7,8] and polystyrene [9]. However, RPC is not suitable for retaining or separating highly polar molecules; normal-phase chromatography (NPC), where the stationary phase is more polar than the mobile phase, is designed for such separations [10]. Typical monolithic stationary phases for NPC are composed of silica or silica modified with amino, cyano and diol functional groups [11–13]. However, such silica-based media possessed the problems of surface chemical heterogeneity and high sensitivity to moisture or polar impurities in mobile phases, which result in poor batch-to-batch reproducibility. Therefore, the combined use of high-purity silica monolith and high-quality solvent was usually required for improved NPC separation.

Polar organic polymer-based monoliths are expected to overcome these problems. Preparation of such monoliths usually involves incorporation of polar functionalities into the monolith backbone by direct copolymerization or post-modification, such as hydroxyl [14,15], epoxy [16,17], diol [18], amino [19], cyano [20], and amphiphilic or zwitterionic groups [21–23]. These polar monoliths have been used in organic or aqueous NPC separation of various polar compounds.

Polyethylene glycol (PEG) is a hydrophilic, non-ionic polymer with chemical structure HO-($CH_2$-$CH_2$-O)$_n$-H, and has been extensively investigated as a material to prevent biofouling [24,25]. PEG macromonomers are molecules composed of a polymerizable moiety connected to a short oligo(ethylene glycol) chain. Recently, free-radical polymerization of PEG macromonomers yielded monoliths that demonstrated size exclusion chromatography (SEC) [26] and hydrophobic interaction chromatography (HIC) of proteins [27] using pure aqueous mobile phases.

Despite their great potential for protein separation, the PEG-functionalized monoliths have never been used for small molecules. The aim of this paper is to show for the first time that the

*Abbreviations:* AIBN, 2,2′-Azobisisobutyronitrile; EDMA, Ethylene dimethacrylate; EtOAc, Ethyl acetate, HETP, height equivalent to a theoretical plate; HIC, Hydrophobic interaction chromatography; ISEC, Inverse size-exclusion chromatography; IPA, Isopropanol; NPC, Normal-phase chromatography; PEGMEMA, Poly(ethylene glycol) methyl ether methacrylate; RPC, Reversed-phase chromatography; RSD, Relative standard deviation; SP, Swelling propensity; TMSPMA, 3-(Trimethoxysilyl)propyl methacrylate

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PEG-functionalized polymer monolith provide a promising alternative when used as a stationary phase for capillary NPC of small molecules. The physical properties of the monolithic column, such as permeability, mechanical stability and porosity, were characterized. Furthermore, this paper first investigated its dual retention properties that explain why the PEG-functionalized monolith could operate in SEC, HIC and NPC modes.

## 2. Experimental

### 2.1. Chemicals

Poly(ethylene glycol) methyl ether methacrylate (PEGMEMA, Mn 300 and 475) and 3-(trimethoxysilyl)propyl methacrylate (γ-MAPS, 98%) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Ethylene dimethacrylate (EDMA) crosslinker and 2,2′-azobisisobutyronitrile (AIBN) initiator were from Acros Organics (Geel, Belgium). To remove inhibitor, EDMA was extracted with 10% aqueous sodium hydroxide and water; after drying over $MgSO_4$, it was filtered and distilled under reduced pressure. Due to its high boiling point, PEGMEMA was purified by passing it through a bed of basic alumina. All test analytes (including thiourea, acrylamide, toluene, phenol, nitrobenzene, and aniline) were ACS (the American Chemical Society) or higher grade. Tetrahydrofuran (THF), HPLC-grade methanol and acetonitrile (ACN) were obtained from J&K Scientific (Beijing, China). HPLC-grade hexane (95% n-hexane) was from J.T. Baker (Philipsburg, NJ, USA). HPLC-grade ethyl acetate and 2-propanol were purchased from Tianjin Kermel Chemical Reagent Co. (Tianjin, China). The water used in all experiments was deionized water from a Milli-Q system (Millipore, Billerica, MA, USA).

### 2.2. Preparation of the monolithic column

The polyimide-coated fused silica capillaries (75 μm i.d. × 375 μm o.d.) were purchased from Yongnian Optical Fiber Factory (Yongnian, Hebei, China). Fused-silica capillaries were silanized using γ-MAPS in order to anchor the polymer monolith on the capillary wall as described previously [26]. The polymer precursor solution was prepared by mixing 0.004 g AIBN, 0.30 g PEGMEA, 0.10 g EDMA, 0.65 g cyclohexane and 0.05 g cyclohexanol using a vortexer. The solution composition corresponded to 36.2 wt% (weight percent) monomers, 63.4 wt% porogens, and 0.4 wt% initiator (1 wt% with respect to monomers). After sonication, the resulting solution was degassed using a stream of nitrogen gas for 5 min before it was introduced into the pre-treated capillary. Both ends of the capillary were sealed with a GC septum and the capillary was heated in a GC oven at 65 °C for 24 h. The capillary column was then rinsed with methanol thoroughly after polymerization in order to remove the porogenic solvents and any other unreacted monomers. For the resulting capillary column, a section containing monolithic polymer was cut and placed on a sticky carbon foil, which was attached to a standard aluminum specimen stub for characterization by scanning electron microscopy (SEM, FEI Quanta 200 ESEM FEG, Hillsboro, USA).

### 2.3. Capillary liquid chromatography

An Ultimate 3000 high pressure gradient LC system (Dionex, Sunnyvale, USA) equipped with an FLM-3300 nano-flow manager (1:1000 split ratio) and operated with Chromeleon software was used for chromatography. The column eluents were monitored using a UV detector equipped with a 3 nL capillary flow cell (ULT-UZ-N10; capillary i.d., 20 μm). The capillary monolithic column was fitted directly into the body of a micro valve injector on one side and connected to the detection cell on the other, using a zero dead volume P-720 Union (Upchurch, Oak Harbor, WA) for minimal post-column dispersion. The sample was injected through a six-port electronic valve (two-position) as injector with 1 μL capillary injection loop, and partial loop (timed) injection was controlled by switching the valve. All experiments in this study were carried out under isocratic conditions. For NPC, the UV detection wavelength was set at 254 nm, the mobile phases were mixtures of hexane with tetrahydrofuran, ethyl acetate, or isopropanol, and the test sample was a mixture of toluene (0.2 mg/mL), nitrobenzene (0.2 mg/mL), phenol (0.2 mg/mL) and aniline (0.2 mg/mL). When switching from RP to NP, the entire system was washed with water, methanol and finally isopropanol. During switching, the pump piston seals also had to be changed.

For investigating the retention properties of the poly (PEGMEMA-co-EDMA) monolith (17 cm × 75 μm i.d.), the UV detection wavelength was set at 214 nm, the mobile phase was an ACN/$H_2O$ mixture, and the test sample was a mixture of toulene (0.1 mg/mL), thiourea (0.1 mg/mL) and acrylamide (0.2 mg/mL). The experiments were carried out with a flow rate of 0.1 μL/min and a sample injection volume of 60 nL. The retention factors of three test compounds were recorded when varying the content of ACN in the mobile phase from 35% to 95%.

### 2.4. Pressure drop measurements

To investigate the permeability and rigidity of the monolithic columns, pressure drop measurements were made at room temperature (~23 °C) using various solvents as permeating fluids at flow rates ranging from 50 to 250 nL/min. The measurements were performed using the same capillary LC system. When not in use, the monolithic column was stored in a methanol/water (80/20, v/v) mixture at room temperature.

### 2.5. Inverse size-exclusion chromatography (ISEC)

ISEC utilizes a set of molecular probes with widely varying, but well-defined sizes to determine pore dimensions. This examination is analogous to molecular mass calibration in SEC. The slope of the SEC calibration curve provides information related to the practical pore size distribution [28]. The same liquid chromatographic system as described in Section 2.3 was used for ISEC. The mobile phase was THF and detection was made at 254 nm. Polystyrene standards with narrow molecular weight distribution and average molecular masses of 201, 2460, 6400, 13200, 19300, 44100, 75700, 151500, 223200, 560900, 1045000, 1571000 and 1877000 were purchased from Scientific Polymer Products (Ontario, NY, USA). Solutions of 0.3 mg/mL polystyrene and toluene each in THF were prepared.

## 3. Results and discussion

### 3.1. Monolith preparation

Choice of porogen is very important in generating the desired porous properties and separation performance of monolithic columns. Typically, a good solvent for both monomer and polymer serves as a microporogen to provide high surface area, while a poor solvent for the polymer provides larger through-pores for bulk flow. For thermally initiated polymerization, high boiling point solvents are often preferred for their low volatility. EDMA is a highly reactive crosslinker containing an ethylene bridge, which provides good flexibility. In contrast, PEGMEMA (Mn 300 or 475) is a more polar monomer containing a longer PEG chain (4/5 or 8/9 ethylene oxide units). Monoliths with smaller pores were usually formed from polymerization mixtures rich in PEG

macromonomer. It is likely that the polarity of PEGMEMA affects the phase separation process that is responsible for creation of the macroporous structure. From empirical porogen screening, only hexane, cyclohexane, and cyclohexanol yielded porous materials. The use of cyclohexane as the only porogen resulted in a very loose structure and very permeable monolith. Introduction of cyclohexanol produced small pores, resulting in increased flow resistance. A mixture of cyclohexanol and cyclohexane was effective in producing defined porous polymers from PEG monomers. In contrast, the use of other solvent mixtures (i.e., cyclohexane with 1-propanol, decanol, or dodecanol) led to nonporous transparent or translucent monoliths.

To increase monolith hydrophilicity, PEGMEMA (Mn ~475, with 8/9 ethylene oxide units) or poly(ethylene glycol) dimethacrylate (Mn ~550, with 12 ethylene oxide units) were also evaluated for preparation of monoliths (based on the same molar concentrations). However, an increase in PEG chain length either in the monomer or crosslinker led to a glassy monolithic structure that did not allow through-flow. To achieve the same objective, we increased the amount of hydrophilic monomer up to 0.3:0.1 monomer/crosslinker while increasing the macroporogen/microporogen ratio to 0.65:0.05 to maintain a permeable structure. Despite the large amount of monomer, the monolithic structure remained rigid and homogeneous. Fig. 1 shows scanning electron micrographs (SEMs) of the resulting poly(PEGMEMA-co-EDMA) monolith. A morphology typical to conventional polymethacrylate monolith was obtained. The monolith was attached to the capillary wall, and no cracks were observed.

### 3.2. Physical characterization of the poly(PEGMEMA-co-EDMA) monolith

#### 3.2.1. Permeability

The flow resistance of any column is conveniently characterized by the column permeability using Darcy's equation [29],

$$k = \frac{uL\eta}{\Delta p}, \quad (1)$$

where $u$ is the fluid linear velocity, $L$ is the column length, $\eta$ is the fluid viscosity, and $\Delta p$ is the column back pressure. $K$ can be estimated by monitoring the flow rate dependence on applied pressure.

Using pressure drop vs. flow rate measurements as shown in Fig. 2, the calculated $K$ values for water, acetonitrile, methanol and n-hexane as mobile phases were determined to be approximately $8.75 \times 10^{-15}$, $5.85 \times 10^{-15}$, $1.13 \times 10^{-14}$, and $2.86 \times 10^{-14}$ m$^2$, respectively. Although the permeability values are lower than those of monolithic silica columns, they are still within the reasonable range for polymethacrylate-based polymer monoliths ($0.15 \sim 8.4 \times 10^{-14}$ m$^2$) [30]. Moreover, compared with particulate columns, its permeability in acetonitrile is comparable to that of 4-μm silica particulate columns [30]. In addition, the column permeability in different mobile phases was different, which demonstrated that the polymer column had swelling phenomena in organic solvents. With nonpolar organic mobile phases (hexane), the poly(PEGMEMA-co-EDMA) monolith was much more permeable than with aqueous and polar organic mobile phases; the addition of 40% polar solvents (THF and IPA) to hexane only produced a small decrease in permeability. However, the column permeability was almost unchanged with the addition of 40% EtOAc to hexane. As a result, the curves for hexane and 40% EtOAc in hexane almost coincided with each other. The hydrophilic nature of PEG hinders swelling in nonpolar solvents like hexane, but helps swelling in polar solvents like methanol, acetonitrile and water. As expected, less swelling polymers had higher permeabilities.

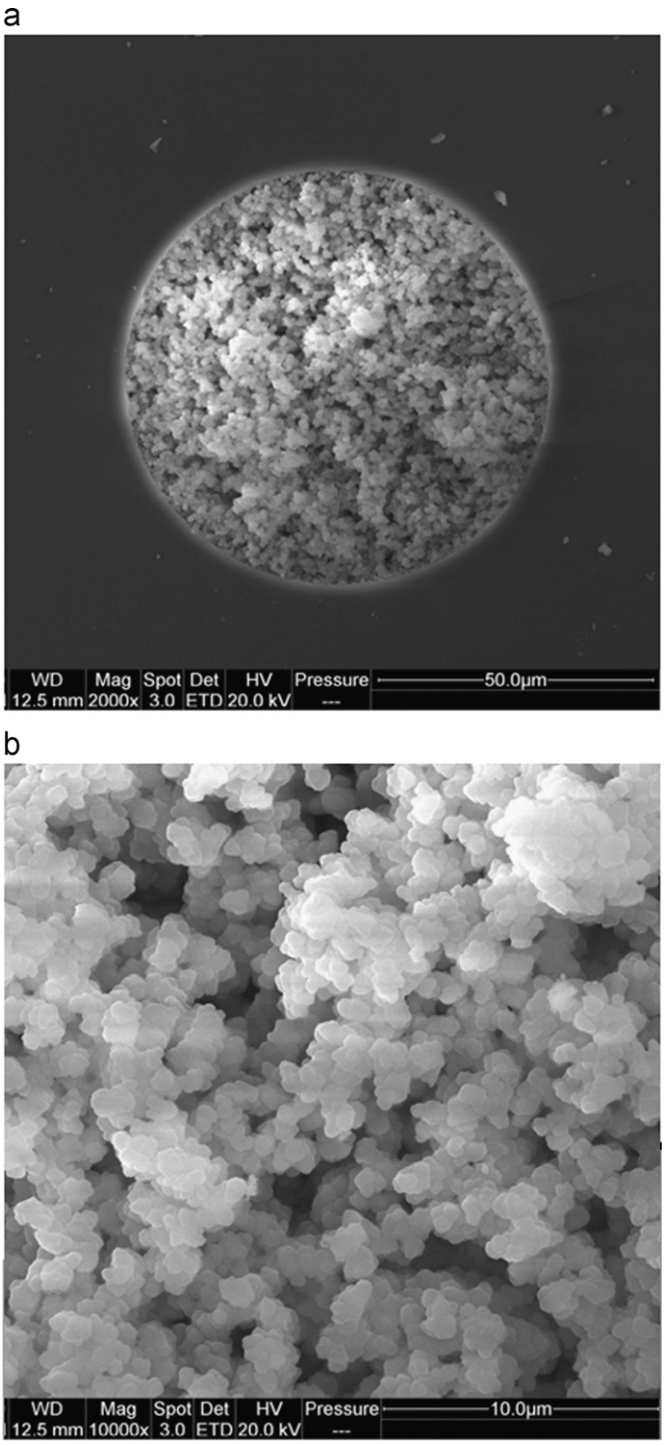


**Fig. 1.** SEM images of the poly(PEGMEMA-co-EDMA) monolithic column with magnification of (a) 2000, (b) 10,000.

#### 3.2.2. Mechanical stability

It is well known that polymer-based stationary phase materials can be adversely affected by organic solvents. The swelling behavior of organic materials can lead to problems such as poor column stability, which leads to reduced chromatographic efficiency and loss of resolution. The swelling propensity (SP) factor is a measure of the shrinkage and swelling of materials in different solvents. To determine the SP values, organic solvents were compared to deionized water at the same flow rate. SP factors were calculated according to the method of Nevejans and

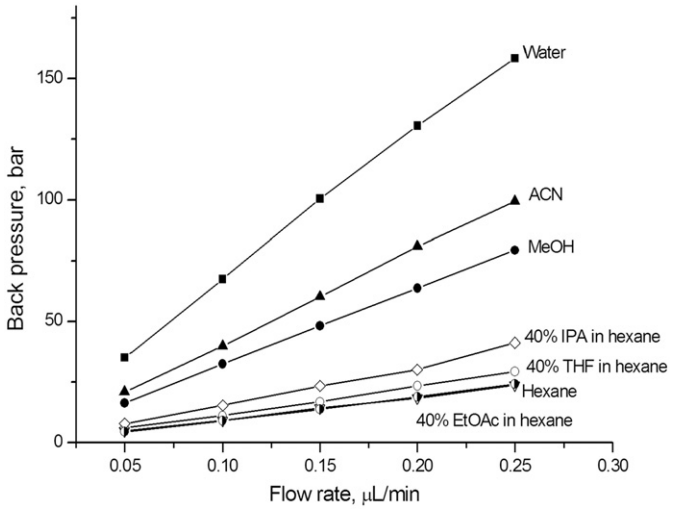


**Fig. 2.** Back pressure dependency on flow rate for a 18 cm × 75 μm i.d. poly (PEGMEMA-co-EDTA) monolith.

Verzele[31]

$$SP = \frac{p(solvent) - p(H_2O)}{p(H_2O)}, \tag{2}$$

where $p = P/\eta$ is the pressure relative to the mobile phase viscosity and P is the column inlet pressure when solvent or water was used as mobile phase. By definition, the closer the value of the SP factor comes to zero, the less the shrinkage problem. For determining SP values, water was used as mobile phase for 10 min, and the pressure drop was measured. Then the mobile phase was switched to an organic solvent and the pressure drop was measured again, once the system was stable. The $\eta$ values for water, ACN, MeOH, and n-hexane at room temperature are approximately $1.00 \times 10^{-3}$, $0.37 \times 10^{-3}$, $0.59 \times 10^{-3}$, and $0.31 \times 10^{-3}$ Pa · s [32]. The SP factors determined for the monolith with ACN, MeOH and n-hexane were 0.62,–0.13 and -0.54. These values are relatively low and indicate that the monolith is rigid; where other polymer monolithic materials reported have SP values ranging from 0.7–1.2 [33–35], and 3.2–37 [31] for some conventional packing materials. Silica-based substrates are hard and typically have SP values of 0.05 [31]. Low swelling tendency of a monolithic polymer material is a basic requirement for its HPLC applicability, since polymer swelling influences the column permeability of the support.

In addition to measuring SP values, the rigidity of the new monolithic phase was evaluated by the pressure drop vs flow rate test for different solvents. Fig. 2 shows plots for a 18 cm × 75 μm i.d. monolithic column. Water, acetonitrile, methanol, n-hexane, and 40% IPA in three solvents (i.e., THF, EtOAc, and n-hexane) were used as mobile phases. These solvents were pumped through the monolith at several flow rates, during which the pressures were recorded. Good linear responses between back pressure and linear flow rate were observed, which clearly demonstrates that the monolith was mechanically stable and capable of withstanding pressures up to 150 bar.

Unlike silica monolith, organic polymer monolith itself provides very good pH stability. The polymer monoliths in our study were prepared in fused-silica capillaries. As we know, fused silica degrades rapidly beyond pH 8. Good coverage by the monolithic polymer at the fused-silica surface can avoid its rapid degradation at high pH values. As shown in Fig. 1(a), SEM image of the polymer monolith inside capillary illustrated that good surface coverage was achieved. Furthermore, the capillary column was tested at low and high buffer pH values (2.0 and 11.0, respectively) over more than several hours and no collapse of stationary phase was observed, showing a good chemical stability. The thermal stability of the monolithic capillary column was also investigated by heating it up to 150 °C and then observing its structure under a microscope. It was found that the crack or deformation of the PEG-functionalized polymer monolith happened beyond 80 °C. Therefore, for long term stability, the PEG-functionalized polymer monolith needs to be stored in an aqueous environment at room temperature.

### 3.2.3. Porosity characterized by ISEC

Since the monolithic material in this study was used in combination with liquids in LC, determination of their pore properties in the wet state should be more valuable than properties measured in the dry state. ISEC provides a convenient method to use, since it is based on LC. Guiochon and co-workers [28] were among the first to use ISEC to characterize the porous structure of silica monolith. They defined several terms to describe the structure of a monolithic bed, such as total porosity ($\varepsilon_t$), external porosity ($\varepsilon_e$) and internal porosity ($\varepsilon_i$) as follows:

$$\varepsilon_t = \frac{V_t}{V_g}, \tag{3}$$

$$\varepsilon_e = \frac{V_e}{V_g}, \tag{4}$$

$$\varepsilon_i = \varepsilon_t - \varepsilon_e = \frac{(V_t - V_e)}{V_g}, \tag{5}$$

where $V_t$ is the retention volume of an unretained tracer, usually assumed to be that of the smallest injected molecule (in this work, toluene (MW=92 g/mol) was used for this purpose), $V_g$ is the geometrical volume of the empty cylindrical column, and $V_e$ is the retention volume of the excluded molecular mass. The excluded molecular mass corresponds to the intersection point of the interpolated straight lines for the internal and external pore zones in the ISEC plot.

In our study, ISEC was carried out using a set of polystyrene standards covering the molecular mass range 201–1,877,000 to determine the porosity. An ISEC plot for the poly(PEGMEMA-co-EDMA) monolith was obtained as Fig. 3(a). The retention volumes were corrected by subtracting the dead volume (~190 nL). From Fig. 3(a), the total porosity ($\varepsilon_t$) was calculated to be 79.2%. The excluded molecular mass was estimated to be 44,100, which corresponds to 33.5 nm. The external porosity ($\varepsilon_e$) was thus calculated to be 69.3% and the internal porosity ($\varepsilon_i$) was 9.9%. The relatively large total porosity (79.2%) accounts for its low flow resistance in hexane.

ISEC also allows the determination of the pore size distribution. $V_n$ is the fractional volume of the pores that have a size equal to or larger than $R_n$, n being the rank of the polymer standard used. Similarly, the fractional volume of the pores with a size equal to or larger than $R_{n+1}$ is $V_{n+1}$ ($R_{n+1} > R_n$). The fractional volume of the pores in the size range between $R_n$ and $R_{n+1}$ is given by:

$$\Delta V_{n+1,n} = V_{R,n+1} - V_{R,n}, \tag{6}$$

where $\Delta V_{n+1,n}$ can be obtained from the ISEC data. In order to relate the molecular mass Mw of a polystyrene sample and the size of the pores from which it is just excluded, the following correlation can be used:

$$M_w = 2.25(10R)^{1.7}, \tag{7}$$

where the pore diameter, $R$, is in nm. Here, by applying Eq. (6) to the retention volumes of the polystyrene standards studies in Fig. 3(a), we can determine the volume of the pores having a range of diameters into which the polystyrene molecules can fit.

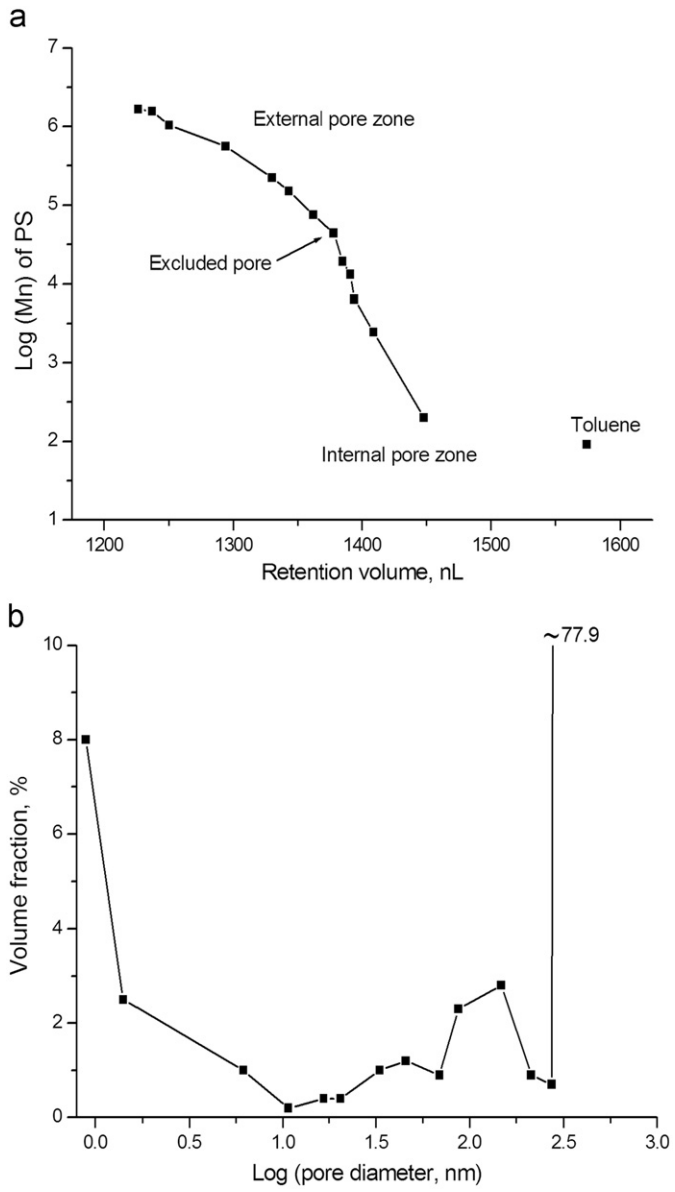


**Fig. 3.** ISEC plot (a) and average pore size distribution (b) for the poly(PEGMEMA-co-EDMA) monolithic column. Conditions: 45 cm × 75 μm i.d. column; THF mobile phase; 0.15 μL/min flow rate; 60 nL injection volume; 254 nm UV detection. Toluene (Mn 92) was used as the smallest probe molecule for determining the total porosity. PS: polystyrene.

As mentioned above, the retention volume of toulene is considered as the total pore volume ($V_t$) since this compound can penetrate into almost all pores. As a result, the volume fraction can be derived by $\Delta V_{n+1,n}/V_t$ (%). Using the obtained data, the pore size distribution was expressed as the logarithm of pore size diameter versus the volume fraction, as shown in Fig. 3(b). A large pore volume fraction (77.9%) of the monolithic column was composed of large macropores having a diameter of 300 nm or larger. This result was in agreement with its large external porosity. In addition, there was a relatively small fraction of pores (8.6%) in the range between 50.0 and 304.2 nm that still qualify as macropores. The pore volume fraction of most mesopores in the range of 1.4∼46.0 nm was 5.4%. The volume fraction of the micropores below 1.4 nm was 8.0%. The volume fraction of the mesopores and micropores was also in agreement with its small external porosity.

### 3.3. Chromatographic evaluation of the monolith

#### 3.3.1. Retentivity in NPLC

In previous studies [26,27], the PEG-functionalized monoliths were hydrophilic in aqueous phases. It was expected that its mild polarity would allow it to perform well in NP chromatography. In order to investigate the NP retentivity of the poly(PEGMEMA-co-EDMA) monolith, the compounds toluene, nitrobenzene, phenol, and aniline were combined in a test mixture. For NPC, a more polar mobile solvent, such as THF, can be added to the mobile phase for increased elution strength. In this study, measurements were performed using typical NP mobile phases composed of n-hexane with THF, ethyl acetate (EtOAc), or isopropanol (IPA). Plots of the retention factors of nitrobenzene, phenol, and aniline as a function of composition of the mobile phase (i.e., THF, EtOAc, and IPA in n-hexane) are shown in Fig. 4. Except for toluene, all other compounds (i.e., nitrobenzene, phenol, and aniline) were well-retained even in a mobile phase that contained as much as 40% strong solvent. This indicates that the poly(PEGMEMA-co-EDMA) monolith can be used with a broad range of mobile phases.

Using n-pentane as a non-retained compound, the average retention factors for the four compounds in 5% THF (IPA and EtOAc) in hexane were 2.3, 1.4, and 4.8, respectively. The average retention values were higher than those measured on bare silica (0.58, 0.17 and 0.58) and diol silica (0.58, 0.17 and 0.64) stationary phases; however, they were lower than those measured for a polymeric phase containing hydroxyl groups (8.6, 6.5 and 7.8), which was in accordance with stationary phase polarity [36].

Fig. 5 shows chromatograms of model compounds using a simple mixture of n-hexane and THF as mobile phase. The elution order was toulene < nitrobenzene < aniline < phenol, which is the same as on bare silica. The elution strength increased with the content of polar modifier. The performance was comparable to that provided by polymeric-diol beads [36]. Polyethers are known to serve as electron donors via the ether oxygen atoms. The polyether oxygen atoms of PEG can serve as hydrogen acceptors to form hydrogen bonding complexes with hydrogen donating analytes [37]. The retention factor for phenol was relatively higher than aniline due to stronger hydrogen-bonding interaction. Here, this hydrogen bonding interaction can be responsible in part for the retention of polar phenols. As shown in Fig. 5(a), using 10% THF in hexane, the resolution between adjacent peaks were calculated to be 3.3, 5.4 and 1.6, respectively. With increasing the amount of THF in hexane, the run time was shortened and the resolution was reduced. Additionally, some peak asymmetry (tailing) was observed in all of separations. We calculated the asymmetry factors at 10% of the peak height. The peak asymmetry factors for toulene, nitrobenzene, aniline and phenol in Fig. 5(a) were 1.4, 1.6, 1.6 and 1.7, respectively. As is well known, while retention in RPLC is based on a partition process, retention in NPLC is based on an adsorption process [38]. Due to the inherent adsorption characteristics, a common problem for separations by NPLC is that polar compounds often show broad tailing peaks. Peak asymmetry causes a decrease in column efficiency and resolution.

#### 3.3.2. Column efficiency

Column efficiency (theoretical plate number) is one of the most important characteristics for evaluation of column performance. Column efficiency depends on a number of factors such as morphology, porosity, homogeneity of the column bed, and flow velocity. The modified van Deemter plot of column efficiency vs linear velocity is often used to illustrate the effects of solute diffusion and mass transfer on the efficiency. Fig. 6 shows the dependency of the height equivalent to a theoretical plate

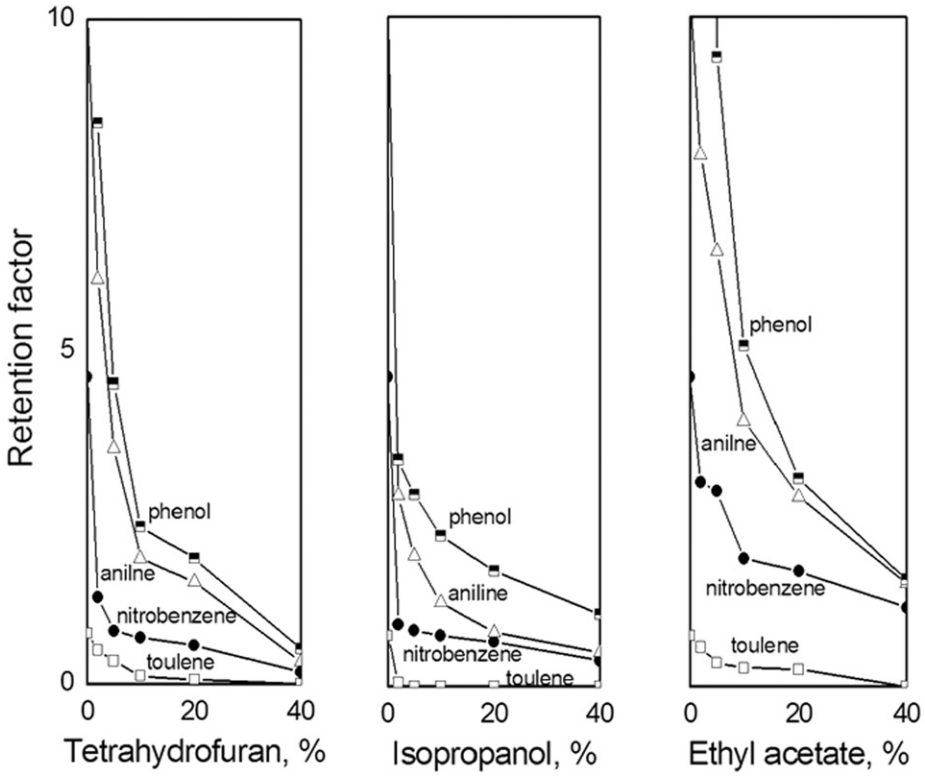


**Fig. 4.** Retention factors of test compounds for different mobile phases. Conditions: 17 cm × 75 μm i.d. column; THF, IPA and EtOAC in n-hexane as mobile phases, respectively; 0.3 μL/min flow rate; 60 nL injection volume; 254 nm UV detection.

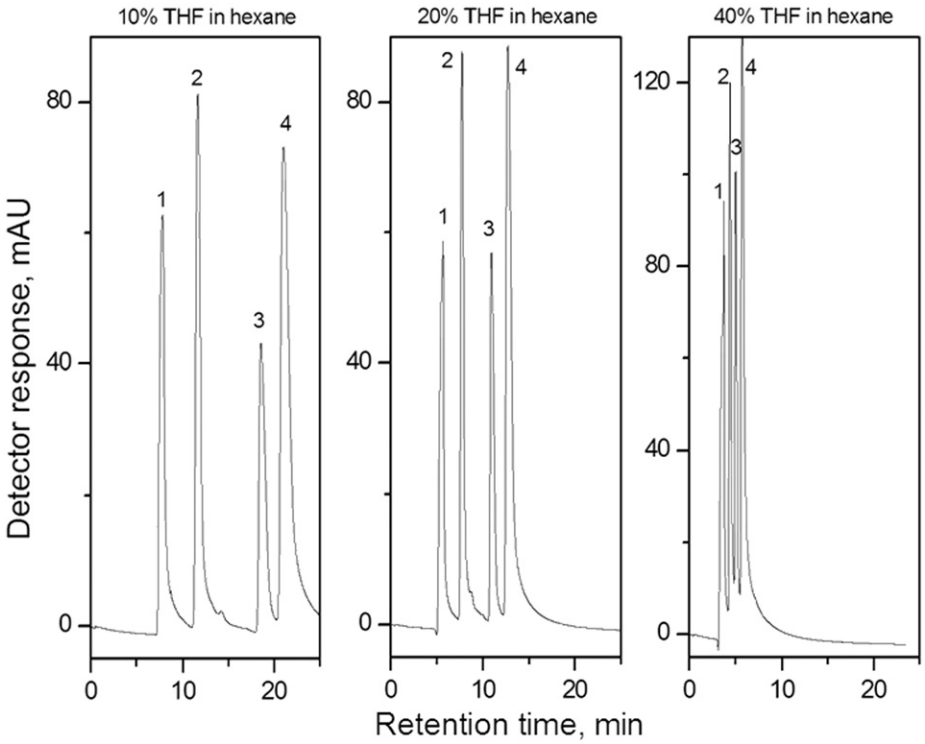


**Fig. 5.** Separation of toluene (1), nitrobenzene (2), aniline (3), and phenol (4) on a poly(PEGMEMA-co-EDMA) monolith using different contents of THF in hexane. other conditions as in Fig. 4.

(HETP, μm) on the linear velocity (mm/s) for nitrobenzene. The van Deemter equation was fitted to the *H-u* curves yielding the three parameters A (14.0 μm), B (9.1 μm mm/s) and C (6.3 ms), which characterize the eddy diffusion, longitudinal diffusion and mass transfer, respectively. Eddy diffusion gave a relatively large contribution to the total plate height due to the inherently inhomogeneous morphology of polymeric monoliths that are produce by a phase separation process. According to the van

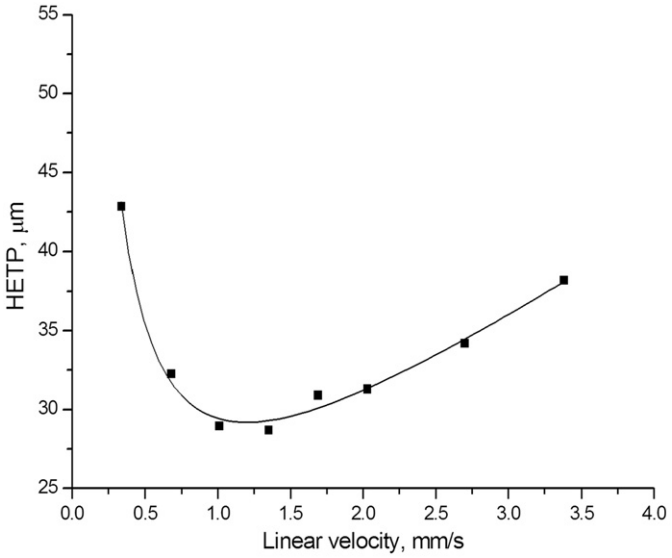


**Fig. 6.** Van Deemter plot for nitrobenzene on the poly(PEGMEMA-co-EDMA) monolithic column. For chromatographic conditions, see Fig. 5, 20% THF in hexane.

Deemter plot, the column efficiency of 33,600–34,320 theoretical plates/m could be achieved at a linear flow velocity of 0.9–1.5 mm/s, which corresponds to a volumetric flow rate of 0.24–0.40 μL/min. The column efficiency obtained was relatively low when compared to 5 μm particle packed column or silica-based monolith. This is a common limitation of polymer monoliths for small molecule separations in isocratic mode because of lack of small pores.

### 3.3.3. Reproducibility

We first investigated run-to-run reproducibility on a single poly(PEGMEMA-co-EDMA) monolithic column by injection of the test mixture. The reproducibility was expressed as the relative standard deviation (RSD) for both retention time and peak area. For three consecutive runs, the RSD values of the retention times for the four compounds were all within 2.3%. The RSD values based on peak areas were lower than 9.2%. In our case, the poor precision of partial loop injection of 60 nL in the 1 μL loop contributed to the deviation a lot [39]. One explanation for the poor precision was that in partial loop injections the sample plug could potentially experience significant dilution. As well known, in normal phase chromatography the retention times are very sensitive to the polar constituents in the mobile phase. The changes in retention could be related to the mobile phase or the accumulation of polar impurities on the column. In our study, this problem could be avoided by using fresh mobile phase or washing the column with an aqueous eluent frequently.

In addition, column-to-column reproducibility measurements gave retention time RSD values ($n=3$) of below 4.2%, indicating a reproducible column preparation. The RSD values based on peak areas were larger (within 10.2%; in most case, less than 8.7%) than those based on retention times since precise injection is difficult in our case. According to our observations, the PEG-based monoliths usually crack or deform during drying, which destroys column performance. Therefore, both ends of the monolithic column were placed in an aqueous environment when not in use. The monolith still appeared dark and homogeneous under the microscope, even after two weeks storage. And no obvious changes in the column back pressure were observed. Additionally, according to our previous study, the PEG-based column was stable for continuous usage at least two months.

### 3.3.4. Dual retention capability

In previous studies, the PEG-functionalized monolith was successfully used in SEC of proteins because it prevented protein adsorption due to its hydrophilicity [26]; The PEG-functionalized monolith was also designed for HIC of proteins due to the presence of both hydrophobic interaction sites and a mildly hydrophilic matrix [27]. In this work, the mild polarity (hydrophilicity) allowed it to perform well in NP chromatography.

According to the molecular formula, the PEG-functionalized monolith was theoretically composed of nonpolar carbon–carbon backbone and multiple PEG side chains. The ether oxygens in the PEG chains formed stabilizing H-bonds with water (hydrophilic), whereas the non-polar carbon–carbon backbone led to competitive hydrophobicity. In fact, the balance between hydrophilic and hydrophobic moieties in the monolith structure was the key parameter that determined its retention properties. In order to investigate its dual retention properties, an ACN/$H_2O$ mixture was used as mobile phase, and the compounds toluene, acrylamide, and thiourea were used in a test mixture. Thiourea is a very polar molecule; as a result, it is well retained in NPC but not retained in RPC.

Fig. 7 illustrates how this dual retention capability works for both polar (acrylamide < thiourea) and nonpolar (toluene) compounds. For the hydrophobic solute, toluene, the retention factor decreased when the ACN content increased from 35% to 85%, and then remained almost constant as the ACN content further increased to 95%, indicating a hydrophobic interaction retention (RP) mechanism. In contrast, while thiourea consistently eluted after acrylamide and toluene, the retention factor of thiourea increased as the ACN content in the mobile phase increased from 73% to 95%. Acrylamide behaved similarly to thiourea, but with much less retention due to its lower polarity. From these results, both reversed phase and normal phase mechanisms could operate simultaneously. The RP mechanism played a main role at low ACN concentrations, while the NP mechanism dominated the retention when using ACN content higher than 73%. From the magnitudes of the retention factors, it is obvious that both of hydrophobic effects and hydrophilic (polar) interactions were relatively weak. In our previous study, due to its good hydrophilicity, the PEG-based monolith could resist non-specific adsorption of proteins even when using an aqueous buffer without any organic solvent additives. Therefore, the SEC mode could be achieved for

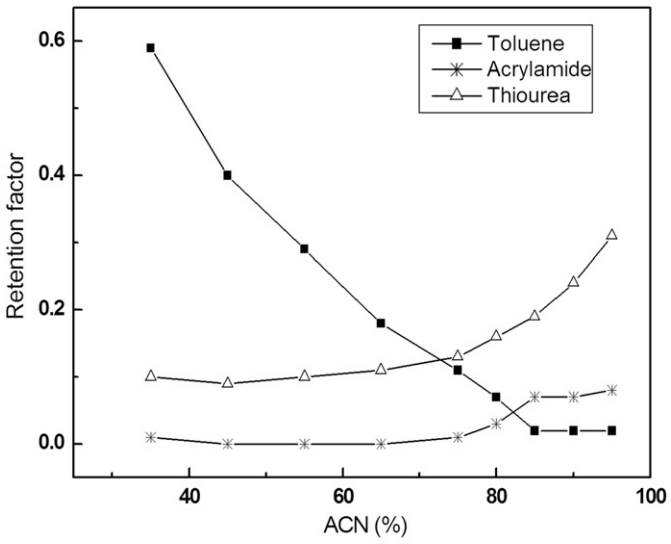


**Fig. 7.** Influence of ACN concentration on retention factors for a poly(PEGMEMA-co-EDMA) monolithic column. Conditions: 17 cm × 75 μm i.d. column; ACN/$H_2O$ mobile phase; 0.1 μL/min flow rate; 60 nL injection volume; 214 nm UV detection.

proteins (or peptides) in aqueous solutions [26]. Although the SEC condition was not referred in this experiment, the hydrophilicity that results in negligible protein adsorption was referred. This elution experiment can explain well why the PEG-functionalized monolith could operate in the various chromatographic modes, including SEC, HIC and NPC modes, by selecting elution conditions.

## 4. Conclusions

A porous poly(PEGMEMA-co-EDMA) monolithic column was prepared by thermal-initiated polymerization in the presence of selected porogens. The chromatographic experiments demonstrated that the hydrophilic, neutral PEG groups can be an alternative functionality that provides obvious normal phase characteristics.

Our results also demonstrated that the PEG-functionalized monolith have a dual retention capability, which makes the various chromatographic modes (SEC, HIC and NPC) possible. This characteristic also allowed retention of both polar and nonpolar compounds when using an isocratic mobile phase. The separation medium was compatible with mobile phases of very different polarities ranging from polar water to non-polar n-hexane. The PEG-functionalized monolith can be an alternative stationary phase for NPLC of small molecules. However, the relatively low polarity and competitive hydrophobic effects make this stationary phase unsuitable for hydrophilic interaction chromatography.

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## References

[1] S. Hjertén, J. Chromatogr. 473 (1989) 273–275.
[2] F. Svec, J.M.J. Fréchet, Anal. Chem. 54 (1992) 820–822.
[3] F. Svec, C.G. Huber, Anal. Chem. 78 (2006) 2101–2107.
[4] H. Minakuchi, K. Nakanish, N. Soga, N. Ishizuka, N. Tanaka, Anal. Chem. 68 (1996) 3498–3501.
[5] N. Tanaka, H. Kobayashi, N. Ishizuka, H. Minakuchi, K. Nakanishi, K. Hosoya, T. Ikegami, J. Chromatogr. A 965 (2002) 35–49.
[6] F. Svec, J. Chromatogr. A 1217 (2010) 902–924.
[7] F. Svec, E.C. Peters, D. Sýkora, J.M.J. Fréchet, J. Chromatogr. A 887 (2000) 3–29.
[8] Z. Jiang, N.W. Smith, P.D. Ferguson, M.R. Tayloer, J. Biochem. Biophys. Methods 70 (2007) 39–45.
[9] A. Premstaller, H. Oberacher, C.G. Huber, Anal. Chem. 72 (2000) 4386–4393.
[10] U.D. Neue, HPLC ColumnsTheor, Technology, and Practicey, Wiley-VCH, New York, 1997, pp. 164-185.
[11] H. Kažoka, J. Biochem. Biophys. Meth. 70 (2007) 15–21.
[12] H. Zhong, Z. El Rassi, J. Sep. Sci. 29 (2006) 2023–2030.
[13] P.A. Sutton, P.N. Nesterenko, J. Sep. Sci. 30 (2007) 2900–2909.
[14] G. Yang, H. Liu, L. Bai, M. Jiang, T. Zhu, Micropor. Mesopor. Mat. 112 (2008) 351–356.
[15] H. Liu, S. Li, L. Bai, G. Yang, Des. Monomers Polym. 13 (2010) 399–406.
[16] K Hosoya, N Hira, K Yamamoto, M Nishimura, N. Tanaka, Anal. Chem. 78 (2006) 5729–5735.
[17] K. Hosoya, K. Mori, M. Sakamoto, T. Kubo, K. Kaya, Chromatographia 70 (2009) 699–704.
[18] H. Zhong, Z.El Rassi, J. Sep. Sci. 32 (2009) 10–20.
[19] P. Holdšvendová, J. Suchánková, M. Bunček, V. Bačkovská, P. Coufal, J. Biochem. Biophys. Methods 70 (2007) 23–29.
[20] A.H. Que, M.V. Novotny, Anal. Bioanal. Chem. 375 (2003) 599–608.
[21] A. Wahl, I. Schnell, U. Pyell, J. Chromatogr. A 1044 (2004) 211–222.
[22] D. Hoegger, R. Freitag, J. Chromatogr. A 1004 (2003) 195–208.
[23] Z. Jiang, N.W. Smith, P.D. Ferguson, M.R. Taylor, Anal. Chem. 79 (2007) 1243–1250.
[24] J.M. Harris, S. Zalipsky, Poly(ethylene glycol): Chemistry and Biological Applications, ACS Symposium Series 680, American Chemical Society, Washington. D.C, 1997.
[25] S.W. Lee, P.E. Laibinis, Biomaterials 19 (1998) 1669–1675.
[26] Y. Li, H.D. Tolley, M.L. Lee, Anal. Chem. 81 (2009) 4406–4413.
[27] Y. Li, H.D. Tolley, M.L. Lee, Anal. Chem. 81 (2009) 9416–9424.
[28] M. Al-Bokari, D. Cherrak, G. Guiochon, J. Chromatogr. A 975 (2002) 275–284.
[29] H. Darcy, in: R.A. Freeze, W. Back (Eds.), Physical Hydrology, Hutchinson Ross, Stroudsburg, PA, 1983.
[30] D. Moravcová, P. Jandera, J. Urban, J. Planeta, J. Sep. Sci. 27 (2004) 789–800.
[31] F. Nevejans, M. Verzele, J. Chromatogr. 350 (1985) 145–150.
[32] M.A. Haidekker, T.P. Brady, D. Lichlyter, E.A. Theodorakis, Bioorg. Chem. 33 (2005) 415–425.
[33] H. Oberacher, A. Premstaller, C.G. Huber, J. Chromatogr. A 1030 (2004) 201.
[34] L. Trojer, S.H. Lubbad, C.P. Bisjak, G.K. Bonn, J. Chromatogr. A 1117 (2006) 56.
[35] W. Wieder, S.H. Lubbad, L. Trojer, C.P. Bisjak, G.K. Bonn, J. Chromatogr. A 1191 (2008) 253.
[36] M. Petro, F. Svec, J.M.J. Fréchet, Anal. Chem. 69 (1997) 3131–3139.
[37] Y. Esaka, K. Yoshimura, M. Gota, K. Kano, J. Chromatogr. A 822 (1998) 107–115.
[38] L.R. Snyder, J.J. Kirkland, J.L. Glajcht, Practical HPLC Method Development, Wiley-Interscience, New York, 1997.
[39] A.D. Jerkovich, R. LoBrutto, R.V. Vivilecchia, LCGC North America (2005) 15–21.