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Polymer monolithic methacrylate base modified with tosylated-polyethylene glycol monomethyl ether as a stationary phase for capillary liquid chromatography

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ABSTRACT

A polyethylene glycol (PEG) monolithic column was successfully prepared in situ for the separation of inorganic anions in ion exchange capillary chromatography. By attaching PEG-groups into the methacrylate-based polymer, the number of theoretical plates was improved from 1433 to 3346 plates/m (when nitrate was used as the analyte). The retention behavior of iodate, bromate, nitrite, bromide and nitrate was observed under various salt aqueous solutions. The retention was based on cations trapped among PEG chain and the positively charged pyridine that work as the anion exchange sites in the PEG monolith. The relative standard deviations (RSDs, for $n=7$) of retention time, peak height and peak area were less than 2.27% for all the analyte anions. The PEG monoliths showed satisfactory mechanical stability and did not swell or shrink significantly with swelling propensity value of 0.34 and 0.64 for methanol and THF, respectively. This stationary phase was successfully applied to the determination of these anions in seawater as well as public drinking water samples.

1. Introduction

Monolithic beds have recently been developed for liquid chromatography (LC) [1]. Their advantages include low column backpressure, simple preparation, requirement of very small amount of stationary phase for a single column, wide application, no use of retaining frits to hold the stationary phase and ease of modification. The porous monoliths consist mesopores and through-pores, which makes them highly porous compared to conventional particle-packed columns. Consequently, monolithic column can conduct the eluent with high flow rate. Basically, monoliths can be synthesized from inorganic material such as silica [2–5] or organic materials such as polymer [6,7] that contain a cross-linking agent, monomers and some porogen in a column tube by in-situ polymerization.

Polymer monolithic stationary phases for ion exchange chromatography (IEC) were fabricated and the separation efficiency was investigated in this study. The fabrication of the polymer monolith usually involves a reactive moiety of the monolith; in this case epoxy group was used, which is then directly modified with functional groups that contain ion-exchange property, such as amino groups [6].

Polyethylene glycol (PEG) is a hydrophilic macromolecule, which only shows mild hydrophilic interaction with proteins at higher salt concentration, and hardly affected the bioactivity of protein under adequate protein purification condition [4]. PEG-functionalized polymer monolith provides the stationary phase for the separation of large and small molecules [6,8]. PEG have been used for stationary phase in reversed phase liquid chromatography. Beside hydrophobic interaction, PEG could also be used for the separation of anions even though PEG does not possess any ion exchange site. Several packing materials which were physically coated and chemically bonded by PEG for IEC have been developed while materials in the form of monolith have been very limited. Rong et al. developed and examined the PEG stationary phase by physically coating for ion chromatography. Lim et al. and Takeuchi et al. have successfully examined the PEG bonded stationary phase for separation of inorganic anions [9–12]. Linda et al. has successfully reacted the PEG with primary amino groups of an aminopropylsilica packing column for separation of inorganic anions [13]. The PEG moiety could form a helix-like conformation in the organic-aqueous media. The separation of anions based on trapping the eluent cations fixed on the oxygen atoms of the PEG chain by ion–dipole interaction that work as the anion-exchange site for separation of anions [14]. Furthermore, the trapping of the

Abbreviations: GMA, glycidyl methacrylate; EDMA, ethylene dimethacrylate; AIBN, 2,2′-azobisisobutyronitrile; Tosylated-PEG, tosylated-polyethylene glycol monomethyl ether; THF, tetrahydrofuran; RSD, relative standard deviation; SEM, scanning electron microscopy; HETP, height equivalent to a theoretical plate; γ-MAPS, 3-(trimethoxysilyl) propyl methacrylate; SP, swelling propensity; IEC, ion exchange chromatography

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Table 1
Various polymerization conditions.

Column	Monomer[a] % (v/v)	Crosslinker[b] % (v/v)	Porogen[c] % (v/v)	Mixture ratio[d]	Modification condition[e]	N_{Max} (plates/m)
M1	7.5	2.5	90	10/90	75 °C, 6 h	*
M2	15	5	80	20/80	75 °C, 6 h	3346
M3	22.5	7.5	70	30/70	75 °C, 6 h	5275
M4	30	10	60	40/60	75 °C, 6 h	1558
M5	17.5	2.5	80	20/80	75 °C, 6 h	4383
M6	12.5	7.5	80	20/80	75 °C, 6 h	3076
M7	10	10	80	20/80	75 °C, 6 h	1389
M8	15	5	80	20/80	75 °C, 4 h	2944
M9	15	5	80	20/80	75 °C, 5 h	3218
M10	15	5	80	20/80	75 °C, 7 h	*
M11	15	5	80	20/80	75 °C, 8 h	*

* Not applicable.
[a] Glycidyl methacrylate (GMA) was always used as a monomer.
[b] Ethylene dimethacrylate (EDMA) was used as a cross-linker.
[c] Three kind of alcohols i.e. ethanol, 1,4-butanediol and decanol were used as porogen.
[d] The volume ratio of monomer+cross-linker to porogen.
[e] 1,4-Dioxane and pyridine were used as the solvents of tosylated-PEG during the modification.

cations on the PEG is similar with trapping on the crown ethers since crown ethers are cyclic PEG, but the former is more flexible [9]. The PEG monoliths have been made and synthesized from PEG-functionalized monomers or cross-linkers for size-exclusion, cation-exchange, and anion-exchange chromatography. Monolithic columns have higher permeability than particle packed columns, which lead to rapid separation of analytes. However, PEG monoliths for the separation of anions have been little reported in the literature. The focus of this study is a polymeric monolith, which requires shorter fabrication time and more robust over a wide range of pH compared to silica-based monoliths. PEG was introduced into the monolith using pyridine will be prepared to improve the column efficiency in the separation of anions since for the PEG stationary phase bromide and nitrite coelute. The PEG monolith was then used as the stationary phase for the rapid and direct determination of inorganic anions in seawater sample as well as public drinking water sample in capillary ion chromatography.

2. Experimental

2.1. Chemicals

Glycidyl methacrylate (GMA) (97%) and ethylene dimethacrylate (EDMA) (97%) were obtained from Wako while *n*-decyl alcohol, 3-(trimethoxysilyl)propyl methacrylate (γ-MAPS, 98%) and 2,2′-azobisisobutyronitrile (AIBN) were obtained from TCI (Tokyo, Japan). 1,4-Butanediol, pyridine and 1,4-dioxane were obtained from Nacalai Tesque (Kyoto, Japan). Polyethylene glycol monomethyl ether p-toluenesulfonate (tosylated-PEG, M.W.1000) was obtained from Aldrich (Rockford, IL, USA). Potassium chloride was of extra pure reagent grade (Nacalai Tesque). Purified water was produced in the laboratory by using a GS-590 water distillation system (Advantec, Tokyo, Japan). All the solutions used in this study were prepared from extra pure reagents obtained from Nacalai Tesque.

2.2. Preparation of monolithic column

The fused silica capillary tube (0.320 mm i.d. × 0.450 mm o.d.) was purchased from GL Sciences (Tokyo, Japan). Fused silica capillary tube was washed using 1 M NaOH solution, deionized water and 1 M HCl in sequence. 30% (v/v) of γ-MAPS in acetone was used for providing methacrylate groups on the inner wall surface of the capillary tube; the tube was sealed at both ends and thermally treated in a water bath at 60 °C for 24 h. Thereafter, the capillary tube was washed with acetone and dried using nitrogen gas for 30 min. As shown in Table 1, mixture solutions of monomer, cross-linker and porogen were prepared at various compositions. Then, 0.2 mL of the solution was completely mixed with 2 mg of AIBN, i.e. the polymerization initiator. The mixture solution was then subjected to ultrasonic vibration for 5 min before it was injected into the pretreated capillary tube. Thermal polymerization was carried out in the water bath at 60°C for 24 h. The capillary tube was rinsed with methanol thoroughly after polymerization to remove unreacted reagent and porogenic solvents. The morphology of the monolith was examined by using scanning electron microscopy (SEM; S-4800, Hitachi, Tokyo, Japan).

2.3. Modification of glycidyl methacrylate base

Subsequently, polyethylene glycol (PEG) group was attached into the glycidyl methacrylate monolith using polyethylene glycol monomethyl ether tosylate (tosylated-PEG). Tosylated-PEG was dissolved in 0.5 mL of 1,4-dioxane and pyridine (50/50, v/v). The solution was passed through to the glycidyl methacrylate monolith and the reaction was carried out by heating in the oven at 75 °C for 4–8 h, followed by washing with methanol at a flow-rate of 4 μL/min for 2 h.

2.4. Capillary liquid chromatography

The chromatographic separation was carried out using a capillary LC system constructed by an L.TEX-8301 Micro Feeder (L.TEX corporation, Tokyo, Japan) equipped with an MS-GAN 050 gas-tight syringe (0.5 mL, Ito, Fuji, Japan) as a pump, a Model 7520 (Rheodyne, Cotati, CA, USA) injector with an injection volume of 0.2 μL, a 100 mm × 0.32 mm i.d. of microcolumn and a UV-1575 intelligent UV/vis detector, (JASCO, Tokyo, Japan) that was operated at 210 nm. The data was acquired using a Chromatopac C-R7A data processor (Shimadzu, Kyoto, Japan). The inlet pressure was monitored with an L.TEX-8150 Pressure Sensor (L.TEX).

3. Results and discussion

3.1. PEG-monolith preparation

PEG monolith was prepared firstly from polymerization of GMA and EDMA under normal temperature for polymerization condition (60 °C) and was modified using Tosylated-PEG dissolving into 1,4-dioxane and pyridine. The good composition of monolith is a

great importance in production site. Generally, the performance of the monolith column was influenced by ratio of monomer to porogen. Hence, investigated ratio of monomer to porogen is necessary for achieving the best methacrylate base before continuing modification step. Firstly, the ratio of monomer to porogen was mainly investigated by keeping the cross-linker content. The resolution between two anions, i.e., IO_3^- and BrO_3^- is shown in Table 2. The monomer/porogen ratio is 20/80 has the best separation profile of anions with resolution approximately 1.15–1.77. When the monomer content was decreased to 10/90, the resolution value could not be calculated because all the anions appeared at the same retention time. Conversely, when the monomer content was increased up to 30/70 and 40/60, the separation of anions was deteriorated and some of the anion peaks were overlapped each other; it was evidenced by resolution values of 1.00–1.36 and 0.85–1.16, respectively. Less amount of porogen also can cause the tight or fully-packed structure of the monolith in the capillary tube. The morphology of the monolith surface was also observed by using SEM, as shown in Fig. 1. Theoretically, by increasing the ratio of porogen, the through-pores will also be increased; on the other hand, when the ratio of the porogen is less, the surface of the monolith will become fully packed and will thus increase the back pressure. From the four SEM photos, showing the morphology of monoliths with the monomer/porogen ratio is 40/60, 30/70, 20/80 and 10/90, it is clearly shown that the monoliths were attached tightly to the inner-wall of the silica capillary tubes, and the estimation ranges of the monolith skeleton are 1–1.5, 2–2.5, 3–4 and 3.5–4 μm, respectively. The reference monolith M2 was chosen as the starting point to continue preparation of the PEG monolith.

Table 2
Resolution of the analytes for various total monomer to porogen ratios.

Mixture ratio[a]	Resolution[b]
10/90	0
20/80	1.15–1.77
30/70	1.00–1.36
40/60	0.85–1.16

[a] The volume ratio of monomer to porogen.
[b] The resolution between IO_3^- and BrO_3^-.

Additionally, the availability of the monomer GMA in this polymerization, also will take an important part because GMA has an epoxy functioning group. Since GMA is used as the monomer to form the methacrylate base, more reactive epoxy groups will be available for the reaction (during the modification) when the amount of GMA in the mixture solution is increased. The increasing number of epoxy group from GMA in the methacrylate base will also increase the amount of PEG attached into the methacrylate base, and thus has affected the efficiency of the column. On the other hand, EDMA is a highly reactive cross-linker containing ethylene bridge, which provides good permeability. Starting from M2 prepared with 15% of GMA, the availability of epoxy group was investigated. Table 1 shows the efficiency of each column for various contents of GMA in the PEG monolith. These proved that increasing GMA content from 10% up to 17.5% (v/v), the column efficiency also increases from 1389 to 4383, in terms of the theoretical plates/m. Although the theoretical plates/m of each column was not very high compared to the others, the PEG monolith can separate 5 anions completely in a short elution time while maintaining a good resolution.

3.2. Effect of modification conditions

Modification condition also has affected the performance of the elution profile of each column. PEG moiety could form helix-like conformation by adjusting itself in the organic aqueous media. As expected, the solvent which contains the amine functional group

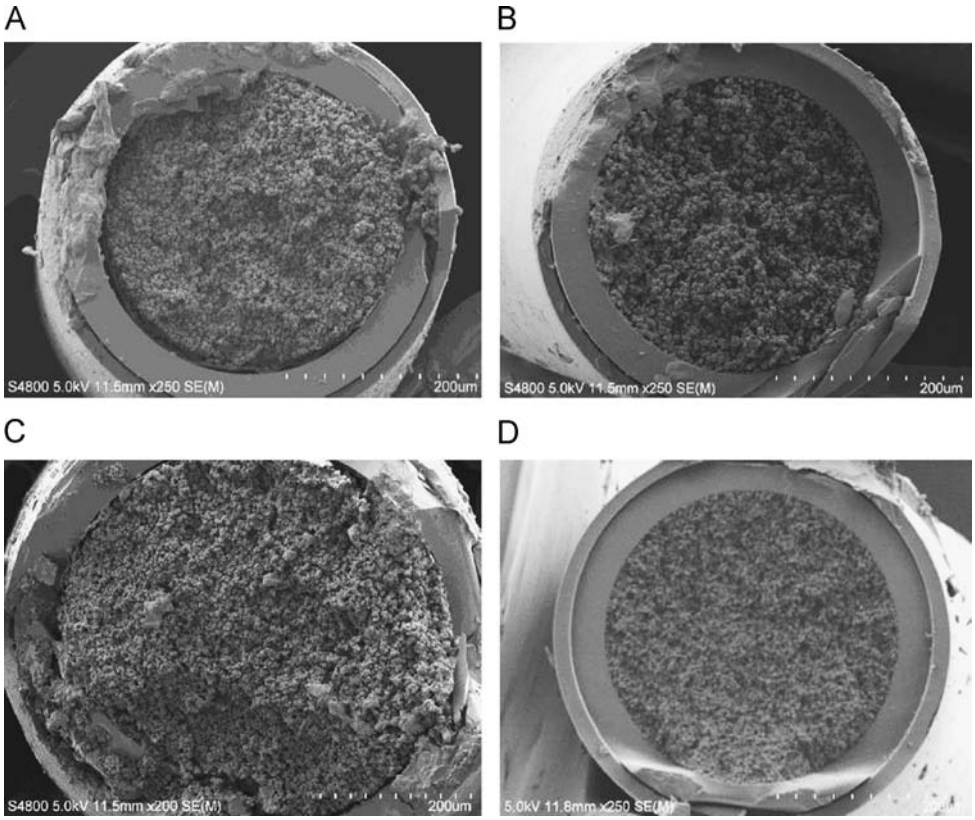

Fig. 1. Scanning Electron Microscope images of PEG-monolithic column. Monomer:porogen is 40/60 (A), 30/70 (B), 20/80 (C) and 10/90 (D).

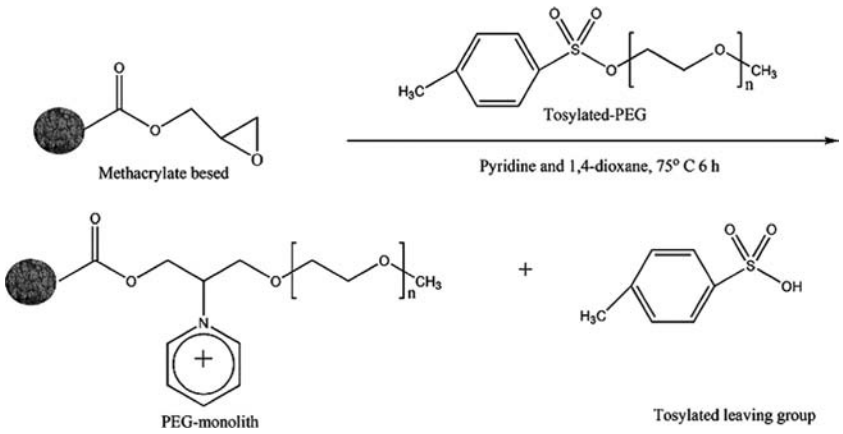

Fig. 2. Schematic diagram of the expected reaction for the preparation of PEG-monolithic column.

could take part in attaching the PEG group into the methacrylate base and the tosylated group from tosylated-PEG modifier would be a leaving group in the modification reaction. The modification of methacrylate base was carried out by attaching tosylated-PEG which was dissolved in 1,4-dioxane and pyridine. The scheme of the expected reaction is shown in Fig. 2. Methacrylate base already has the epoxy groups as reactive sites, firstly the reactive nucleophiles that were introduced by pyridine worked to open the epoxy group and another hydroxyl group from the opened epoxide was reactive for attaching the PEG. Since the nucleophiles are required for attaching the PEG groups, pyridine was chosen in this research. Pyridine has been used as a precursor in some reactions and could be as the supplier of nucleophiles. On the other hand, since tertiary amine in pyridine could work as the anion exchange site, separation profiles were compared between PEG-monolith and monolith without PEG chain. The elution of five inorganic anions on the stationary phase with and without PEG was displayed in Fig. 3. When the monolith was modified with pyridine without attaching any PEG groups, five inorganic anions could not be separated well unlike when monolith was modified with PEG. Hence, attaching PEG groups onto the monolithic column could improve the separation performance of anions compared to without any PEG groups on the column that has 1433 theoretical plates/m for nitrate.

Reaction time will take an important part in modification condition. It was found that the reaction time of attaching the PEG into the methacrylate base affected the profile of separation of anions. Decreasing the reaction time gives longer elution time of each anion. When the reaction time increased from 4 to 6 h, the elution time of the anions become shorter (Fig. S1). Unfortunately, the reason of this fact is not certain. The column efficiency increased from 2944 to 3346 theoretical plates/m for nitrate at a flow rate of 4 μL/min. However, when the reaction was done for 7 and 8 h, the column efficiency could not be evaluated because the peaks of anions were totally broaden, and the elution order of the anions were irregular compared to those obtained at lower reaction times (Fig. S2). This condition might be due to optimum reaction conditions that already achieved and longer modification will only cause damage to some of the reagents used. The separation profiles obtained when modification at lower times was very different than those modified for 7 or 8 h; technical problem (misconduct) during the preparation and modification reaction also likely to be one caused. Attaching the PEG into the methacrylate base at 75 °C for 6 h showed the best separation profile.

3.3. Optimization of eluents

Some salt aqueous solutions such as LiCl, NaCl, KCl, RbCl, CsCl, NH_4Cl, $MgCl_2$, $SrCl_2$ and $CaCl_2$ were used as the mobile phase. The

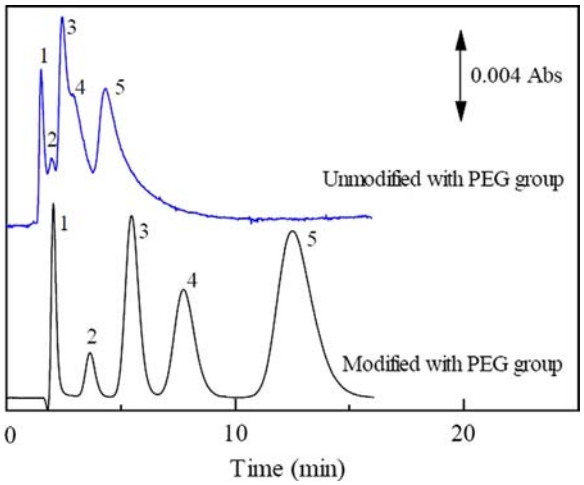

Fig. 3. Separation of inorganic anions on the stationary phase with and without PEG groups. Operating conditions: Analytes, 1 mM of each iodate (1), bromate (2), nitrite (3), bromide (4) and nitrate (5); PEG-monolith M2 column (100 × 0.32 mm i.d.); 100 mM of sodium chloride eluent; 4 μL/min flow rate; 210 nm wavelength of UV detection and 0.2 μL injection volume.

retention time of 5 inorganic anions was affected by the eluent cations which were trapped among the PEG chains and functioned as the anion-exchange sites in the PEG monolithic stationary phase. The positively charged pyridine also works as the anion exchange site in the PEG monolith for the separation of 5 anions. The existence of the positive charge on the pyridine was found to have a positive effect on the separation of anions because the elution order of these anions on the PEG stationary phase was found similar to those observed in the conventional ion exchange chromatography except coeluting of bromide and nitrite. In addition, the eluent cations also affect the retention behavior of the anions because eluent cations were expected to be trapped onto the PEG chains by ion–dipole interaction with the oxygen atom of the multiple PEG chains [14]. Unfortunately, the elution of bromide and nitrite was overlapped for PEG stationary phase. Hence, existence of positive charge on pyridine could solve this problem. Fig. 4 shows the retention behavior of 5 anions using aqueous solution as the eluent. Different eluent cations gave the different elution time of anions. The retention of anions decreased when hydrated cation with smaller size was used as the eluent. In this case eluent CsCl showed the shortest retention of anions, whereas some peaks of the anions were overlapped. Conversely, using the divalent cation i.e. $MgCl_2$, the retention of anions was decreased although the $MgCl_2$ has a bigger size. This could probably be explained by the fact that the retention of anions was not solely

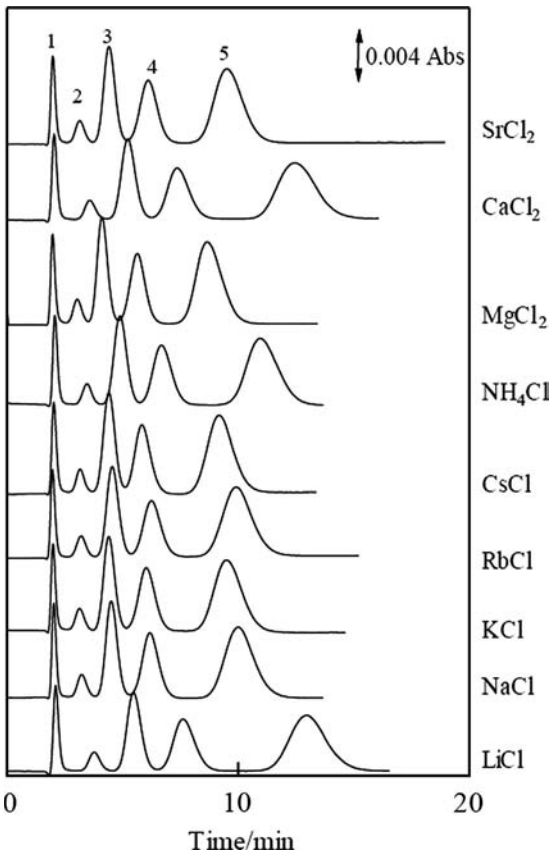

Fig. 4. Chromatograms of inorganic anions with various chloride eluents. Analytes, 1 mM each of iodate (1), bromate (2), nitrite (3), bromide (4) and nitrate (5); PEG-monolith M2 column (100 × 0.32 mm i.d.); 100 mM aqueous solution of various chloride eluent, as indicated; 4 μL/min flow rate; 210 nm wavelength of UV detection and 0.2 μL injection volume.

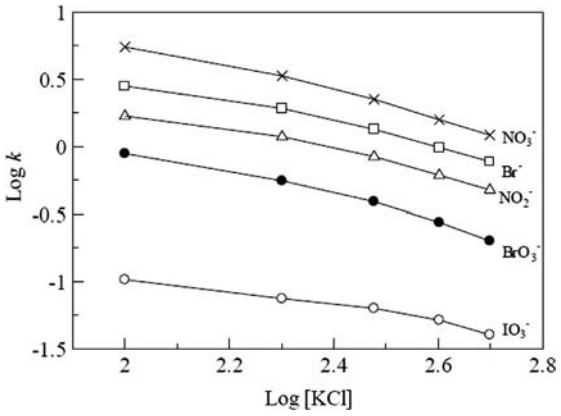

Fig. 5. Effect of potassium chloride concentration (log [KCl]) on retention (log k) of inorganic anions. Analytes, inorganic anions including 1 mM each of iodate (1), bromate (2), nitrite (3), bromide (4) and nitrate (5); PEG-monolith M2 column (100 × 0.32 mm i.d.); 100 mM of potassium chloride eluent; 4 μL/min flow rate; 210 nm wavelength of UV detection and 0.2 μL injection volume.

influenced by the size of ionic radii of the trapped eluent cations [15]. Unlike crown ethers (i.e. cyclic PEG) stationary phases, in which only cations with a specific size are trapped within the cavity of crown ethers, the eluent cations investigated in this study were found to be trapped regardless of their ionic radius. The ionic radii increases in the following order: Li^+ (0.090 nm) < Na^+ (0.116 nm) < K^+ (0.152 nm) < Rb^+ (0.166 nm) < Cs^+ (0.181 nm) for the monovalent cations, and Mg^{2+} (0.086 nm) < Ca^{2+} (0.114 nm) < Sr^{2+} (0.132 nm) for the divalent cations [15]. By right

Table 3
Relative standard deviation of retention time, peak height and peak area for five anions under optimum operating condition as in Fig. 3.

RSD (% $n=7$)			
	Retention	Peak area	Peak height
IO_3^-	0.46	1.79	1.56
BrO_3^-	0.62	2.33	2.02
NO_2^-	0.73	1.28	0.96
Br^-	0.65	2.19	2.27
NO_3^-	0.5	1.00	0.86

the retention of anions should also follow this order, however, irregular retention behaviors were observed. Nevertheless, NH_4^+ with ionic size slightly bigger than K^+ was found having exceptional retention for these anions. On the other hand, $MgCl_2$ showed the shorter retention time than monovalent cations even CsCl, as shown in Fig. 4. Therefore, we could conclude that the retention of anions was not solely affected by the size (ionic radii) of the cations. Unfortunately, the reason for this phenomenon is yet to be elucidated. In Fig. 4, KCl shows the best resolution among the others and thus it was used for the following experiments.

Moreover, the effect concentration of salt was investigated. Fig. 5 shows the logarithm of retention factor (log k) of anions as logarithm function of the eluent concentrations. From the figure it can be seen that the plot of each anion was almost linear and the slopes were −0.52, −0.90, −0.78, −0.80 and −0.94. Theoretically, in this case if ion exchange is involved in the retention of anions, the slopes of each anion should be −1.0 because the analyte anions and the chloride anions are also monovalent. Since the slope for each anion is nearly to −1.0, the coordination of cations which were trapped in the PEG chain could work during separation of anions in ion exchange mode. With increasing concentration of cations in the mobile phase, the coordinated cation also increased and the retention time of anions decreased. The relative standard deviations (RSDs) of retention time, peak height and peak area of five anions were calculated under the same operating condition. The results are provided in Table 3. The RSDs ($n=7$) of retention time, peak height and peak area were less than 2.27% for all the analyte anions. In other words, these values show satisfactory repeatability of the PEG column.

3.4. Mechanical stability

Monolithic column stationary phase should have an excellent mechanical stability and permeability. The mechanical stability of the column can be obtained by calculating the swelling propensity (SP) factor and also by plotting the pressure drop to the flow rate. SP factor is a measure of the shrinkage and swelling of materials in different solvents. To determine the SP value, deionized water and organic solvent were compared at the same flow rate. SP factors were calculated based on the methods of Nevejans and Verzele [16],

$$\mathrm{SP} = \frac{p(\text{solvent}) - p(\text{water})}{p(\text{water})}$$

where p is the pressure relative to the viscosity, $p = P/\eta$. Generally, no swelling occurs in the column if SP = 0 whereas more swelling occurs if the SP value is higher. On the other hand, a negative value indicates shrinkage of the monolith [17]. In this study, the SP value of column M2 was determined by using water as the eluent for 30 min and the pressure drop was marked. Furthermore, eluent was changed to methanol and when the system became stable, and the pressure drop was marked again. THF was also operated in the same way as methanol for determination of SP value. The SP values of monolith M2 were found to be ca. 0.34 and 0.64 for methanol and THF,

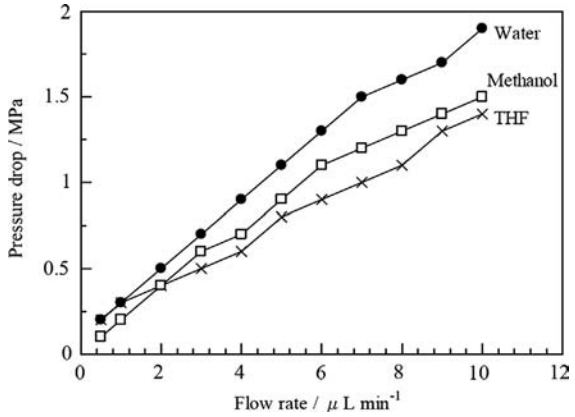

Fig. 6. Back pressure of M2 as a function of flow rate of water, methanol and THF.

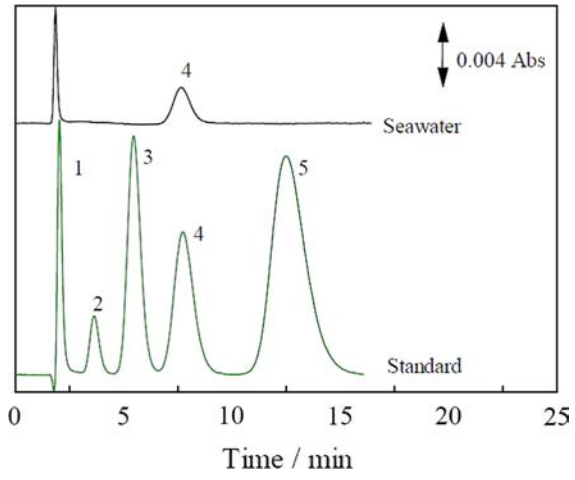

Fig. 7. Separation of anions contained in seawater sample. Operating conditions as in Fig. 3.

respectively. These values showed that there is no significant swelling or shrinkage of the PEG monolith occurred in the capillary. Hence these monoliths could work on different polarities with the high flow rate compared to other polymer monolith materials reported with SP values from 0.16 to 1.1 [1,16,18–19]. Fig. 6 shows a pressure drop plotted against flow rates indicates an excellent linearity with $R^2=0.995$. Although water, methanol and THF were used with flow rate up to 10 μL/min, the pressure drop in the system was still less than 1.9, 1.5 and 1.4 MPa, respectively. These results indicated that the PEG monolithic column could be operated for a fast, efficient analysis with a higher flow rate and with less pressure drop. By plotting the pressure drop against the flow rates, the permeability values for water and methanol used as the mobile phase were calculated to be 8.0×10^{-13} and 5.3×10^{-13} m^2, respectively. These values are relatively higher than the range for polymer methacrylate-based monoliths around $0.15–8.4\times10^{-14}$ m^2 [20].

3.5. Application to real samples

The seawater of Padang beach and the public drinking water collected from Padang city, West Sumatera, Indonesia, were analyzed using the developed column. The seawater and public drinking water samples were applied in this method within a few days after collection. The samples were filtered through a 0.45 μm membrane filter prior to injection. By using this method, bromide could be determined in the seawater with concentration of ca. 44 ppm (Fig. 7) and the nitrate was determined to be ca. 1.9 ppm in the public drinking water (Fig. 8). The detected nitrate is still

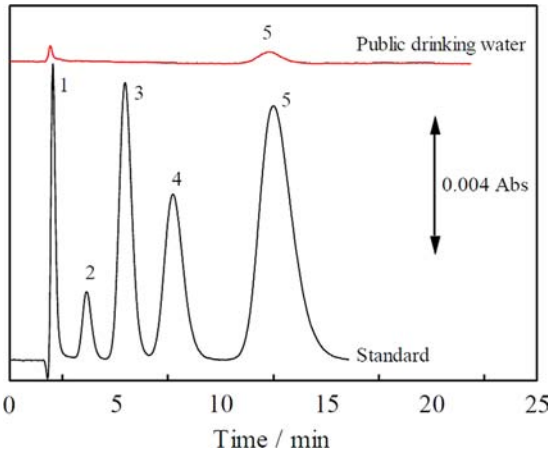

Fig. 8. Separation of anions contained in public drinking water sample. Operating conditions as in Fig. 3.

under the maximum limit of the public drinking water permission in the Padang city, West Sumatera, Indonesia. The anion-exchange capacity of the stationary phase was not high enough to separate the iodate from the water-dip, which disturbed the accurate determination of iodate.

4. Conclusions

A simple and rapid method for analyzing 5 anions using PEG monolithic column which was fabricated via thermal in situ polymerization has been reported. The chromatographic results showed that PEG monolith can be a good alternative for the rapid determination of anions. Introduction of PEG-groups could improve the number of theoretical plates of the monolith compared to those without any PEG-groups. In addition, good mechanical stability was obtained by the fact that there was no significant swelling or shrinkage occurred in the capillary, and the PEG monolith could worked on eluents with different polarities at high flow rates. The present method allows direct determination of anions contained in the seawater sample without any pretreatment. Bromide contained in seawater collected from Padang beach and nitrate contained in public drinking water in Padang City have been determined by this method, and the concentrations were calculated to be 44 and 1.9 ppm, respectively.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.10.060.

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