One-pot Synthesis of Polyethylene glycol-based Polymeric Monolithic Stationary Phases for Capillary Ion Chromatography

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Abstract

Polyethylene glycol-based polymeric monolithic stationary phases (capillary columns of 0.32 mm I.D.) were successfully prepared via one-pot-single-step reaction and were use in ion chromatography for the separation of several common inorganic anions. It should be noted that the prepared polyethylene glycol-monoliths were able to retain and separate the anions even though there were no ion-exchange sites. The retention mechanism was found to be based on the eluent cations trapped among the polyethylene glycol chains and worked as the anion-exchange sites. Several parameters such as the reaction conditions, monomer:porogen ratios, type of eluents, etc., were investigated. The relative standard deviations obtained for the retention times and signal intensities were less than 5 and 10\% respectively. Theoretical plate numbers obtained for the separation of these anions were calculated to be in the range of 700-4300 plates for capillary columns of 10 cm in length.

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Keywords: Capillary ion chromatography, Inorganic anion, Polymer-based monolithic capillary column, Poly(ethylene glycol) methyl ether methacrylate-bonded stationary phase

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**Introduction**

Ion Chromatography (IC), since its first introduction by Small *et al.* in 1975 [1], is a type of liquid chromatography and has been widely used especially for the separation as well as analysis of inorganic cations and anions, and/or small organic ions and amino acids in aqueous samples [2–4]. Over the past 45 years, IC has been developed as a routine analytical method for natural waters, *i.e.* drinking water, seawater, pond and lake water, as well as industrial wastewaters [5] for the monitoring of water quality and its safety for human activities.

Up till now, various types of stationary phases have been developed in IC, and most of them have functional groups with charged or chargeable moieties, where analyte ions have electrostatic attraction and/or repulsion [6]. Among IC columns, silica-gel is ideal support due to its favorable characteristics, such as high chemical and thermal stability, good mechanical strength, controllable surface area and pore structure, surface rich in silanol groups, and so on. On the other hand, polymer-based support appears to be an attractive alternative due to its high pH durability that in favor of sample storage/preparation prior analysis [7].

With the growing demand for high-throughput analysis as well as concern for environmental-friendly routine analytical methods, down-sizing of the separation systems is found to be very effective [8–10]. The shift from “conventional” to “capillary” is getting more popular as it possesses advantages such as less consumption of mobile phase and stationary phase, increased mass sensitivity of the detection, and it is also easier to develop and explore new stationary phase due to only small amount of column material is needed for the capillary [10].

For the case of particle-packed columns, when the particle diameter is reduced, the pressure load of the separation system will increase in inversely proportion to the square of the charged particle diameter. Resulting in higher pressure resistance of the separation system will be required, and this will be a limiting factor in most separation systems. In recent years, monolithic columns have attracted much attention as separation columns due to their high porosity compared to particle-packed columns [8–11].

Similarly, silica-based monoliths, which are prepared through the sol-gel method using alkoxyssillanes, generally show high mechanical strength and excellent separation performance compared to organic polymer-based monoliths, which are prepared by reacting a solution comprising copolymerized monomer(s), a crosslinking agent, porogen(s) (pore-forming agent), and a polymerization initiator [11,12]. The organic polymer-type monolithic columns are easier to prepare. Moreover, they show excellent pH durability, which is more suitable to be used as stationary phases for IC applications [12].

Our research group has proposed the use of polyethylene glycol (PEG) or polyoxyethylene (POE) as the stationary phases for the separation of inorganic anions in IC. Indeed, the retention mechanisms involved in the separation of anions on these PEG- as well as POE-based stationary phases were discussed in details [13]. One of the major advantages of using PEG- or POE-based stationary phase is that we can carry out the direct determination of trace anions in seawater as well as public drinking water samples without the need of tremendous sample pre-treatment process [13–16].

PEG is a well-known polymer of ethylene oxide, and besides hydrophobic interaction, PEG or POE moieties can provide other interactions such as hydrogen bonding and dipole-dipole interaction [13]. Our research results show that chemically bonded PEG and polyethylene oxide (PEO) particle-packed capillary stationary phases could be used for the ion-exchange separation of inorganic anions even though there is no ion-exchange moieties present on the stationary phase [14,15]. For further applications in real sample analysis, polymer-based PEG-monoliths were developed and applied for IC analysis of seawater as well as public drinking water [16].

With the rapid development of new chemicals and the advancement of chemical reactions, the polymerization reactions are becoming easier and more promising results can be obtained. Compared to multiple-step reactions [16], in this study, one-pot-single-step *in-situ* preparation of PEG-based monolithic capillary columns was carried out for the first time, and the synthesized monoliths were evaluated by separating several inorganic anions under IC separation mode. The single-step reaction was possible by using polyethylene glycol methyl ether methacrylate (PEGMEMA) as the monomer, and it was reacted with a traditional cross-linker, *i.e.* ethylene dimethacrylate, together with an initiator (2,2’-azobisisobutyronitrile, AIBN) and biporogenic solvent (1-propanol and 1,4-butanediol).
PEG or PEO, with their interesting non-ionic but amphiphilic property, are expected to provide multiple separation modes within a single column. This study shows the preliminary results of using a commercially available PEG-based monomer for the one-pot-single-step in-situ fabrication of organic polymer-based PEG monolithic capillary columns.

Experimental Section
Materials and Apparatus

All reagents were guaranteed reagent grade and were obtained from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), Tokyo Chemical Industry Co. Ltd (Tokyo, Japan), or Nacalai Tesque (Kyoto, Japan), unless otherwise indicated. PEGMEMA was obtained from Sigma-Aldrich Co. LLC (St. Louis, MO, USA). Lithium chloride, magnesium chloride, and strontium chloride were obtained from Yoneyama Yakuhin Kogyo Co., Ltd. (Osaka, Japan). Purified water was produced in the laboratory by using a simple UV water purification system (Merck Millipore, Darmstadt, Germany).

All experiments were carried out using a capillary liquid chromatography system constructed from the following apparatuses. The pump was a microfeeder model 8301 (L. TEX Corporation, Tokyo, Japan) equipped with a 0.5-mL gas-tight syringe (ITO Corporation, Fuji, Japan). Capillary columns were prepared using fused-silica capillary tubes (100 × 0.32 mm I.D., GL Sciences Inc., Tokyo, Japan). A model 7520 micro injector (Rheodyne, Cotati, CA, USA) with an injection volume of 0.2 µL, and a UV detector model UV-2070 (JASCO Corporation, Tokyo, Japan) was used at a detection wavelength of 210 nm. CDS-Lite Ver. 5.0 (LAsoft LTD., Chiba, Japan) was used to process the data.

Pretreatment of fused-silica capillary tube

Fused-silica capillary tube was washed with 1 M NaOH solution, deionized water, and 1 M HCl in sequence for 30 mins each. 30% (v/v) of 3-(trimethoxysilyl)propyl methacrylate (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 hs. Subsequently, the capillary tube was washed with acetone and dried using nitrogen gas for 30 mins.

One-pot synthesis of PEG-monolithic capillary column

PEGMEMA was mixed with ethylene dimethacrylate (EDMA) together with an initiator (AIBN) and a biporogenic solvent (1-propanol and 1,4-butaneadiol were used in this study) in a single reaction pot. The mixture solution was placed under ultrasonic vibration for 5 mins before it was injected into the pretreated capillary tube. Thermal polymerization was carried out in a water bath at 60 °C for 24 hs. The resulted capillary column was rinsed with methanol in order to remove unreacted reagents and porogenic solvents. The scheme of the expected reaction is shown in Figure 1. The morphology of the monolith was examined by using scanning electron microscopy (SEM; S-4800, Hitachi, Tokyo, Japan).

![Figure 1. Schematic diagram of the expected one-pot reaction for the preparation of PEG-monolithic capillary columns.](https://example.com/figure1.png)

Evaluation of PEG-monolithic capillary column

Separation efficiency of the PEG-monolithic capillary column was evaluated in IC separation mode. Several simple inorganic anions were chosen as the analytes and the separation mechanism of the PEG-monolithic columns was investigated by varying the eluent type and concentration. Separation profiles of the analytes were observed and efficiency was calculated in terms of theoretical plate numbers.

Results and Discussion

One of the main challenges in making monolithic stationary phase is the column...
preparation repeatability [12]. Therefore, the PEG-monolithic capillary columns were prepared (under optimized conditions) and the separation efficiency was evaluated repeatedly in this study.

**Preparation of PEG-monolithic capillary columns**

Due to the huge possible combinations of polymerization conditions, EDMA was used as the cross-linker. Biporogenic solvent (*i.e.* using 2 different organic solvents as the pore-forming agents) was selected for the formation of through-pore as well as meso-pore of the PEG-monoliths. Polyethylene glycol is a hydrophilic macromolecule, therefore 1-propanol and 1,4-butanediol were selected as the porogens [17] in this study.

Various combinations of the monomer, cross-linker and porogens were used for the polymerization, and monoliths were formed when the reaction was carried out for more than 12 h at 60 °C. These monoliths are fabricated in capillaries of 0.32 mm with 10 cm in length, since the resulted amount of products are limited, the capillaries were carefully cut into 2 cm in length and the morphology of the cross-sections of the capillaries were observed using SEM. The polymer structure plays an important role in the separation efficiency, therefore SEM images were used to identify the possible differences of the monolith matrix (back-bone).

Table 1 shows the various combination of the monomer, cross-linker, porogens, as well as the initiator. Generally, the ratio of monomer and cross-linker to the porogens should be kept between 30:70 and 10:90 in order to produce monoliths with high porosity while keeping a good separation efficiency. Specifically, it would be ideal to keep a high content of the monomer in order to have a maximum separation efficiency of the monoliths.

Columns A, B and C were prepared with the porogen contents of 70, 80 and 90%, respectively, and the SEM images of the monoliths are shown in Figure 2, A, B and C, respectively. SEM image of Figure 2-A showed that is no obvious through-pores while Figure 2-B and C showed relatively better morphology. The separation profiles of several common inorganic anions for columns A, B and C are shown in Figure 3. Poor separation degree between samples 1-2 and 4-5 was observed, however, longer retention of most of the anions was observed for column B, therefore 80% of the porogen content was found to be the best for this PEG-monolith. Further experiments were carried out by varying the amount of each porogen while maintaining a total of 80%.

Columns D, E and F were prepared by varying the ratio of the porogens. The percentage of the good solvent P1, *i.e.* 1-propanol, was reduced from 87.5, 75 and 62.5%, for columns D, E and F, respectively, and the relative SEM images are shown in Figure 2D-F, E, and F. Column F was found to have the best monolith morphology.

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1°C-column, 2M=monomer; PEGMEMA, 3CL=cross-linker; EDMA, 4P1=porogen 1; 1-propanol, 5P2=porogen 2; 1,4-butanediol, 6Polymerization time = 24 h, 7Polymerization time = 18 h

![Figure 2. SEM images of the prepared PEGMEMA-monoliths A to F in accordance to the various combinations as shown in Table 1.](image-url)
The reaction time, i.e. polymerization time of the preparation of PEG-monolithic capillary columns was varied from 12 to 48 hs, after several repetitions, on average, the columns obtained from polymerization time at 12 hs did not show good separation of the anions, while polymerization time of more than 24 hs showed relatively good separation of the five anions, however, polymerization time at 48 h showed peak tailing especially for iodide and thiocyanate ions. As shown in Figure 5, monolithic morphology was found to be the best at polymerization time at 18 hs, therefore, the ratio of each porogen was further investigated to obtain the best PEG-monolithic columns.

Figure 4 shows the separation profiles of inorganic anions obtained from columns D, E, and F. As mentioned before, column F showed the best morphology, and this was further proven by the relatively good separation shown in Figure 4-F. Comparing to columns D and E, in which the overlapping of samples was observed, column F was able to separate each anion, and a degree of resolution of more than 1.5 (baseline separation) was observed between samples 3-4 and 4-5. Therefore, the optimum percentage of the good solvent 1-propanol was found to be 62.5%.
Figure 6 shows the SEM images when varying the amount of porogens, i.e. the ratio of good solvent:weak solvent. Columns F-A and F-B were blocked and the eluent could not be passed by. Therefore, the ratio of good solvent:weak solvent was found to be optimum at 5:3 under these conditions, in which the composition is indicated in Table 1 as column F-C.

As for the column reproducibility, after several repetitions, on an average, polymerization time at 12 hs showed insufficient formation of the through-pore, while 90% of the monoliths formed by >24 hs polymerization had overly packed monoliths, causing it not able to be used for separation purposes. 18 hs polymerization time was found to be optimum with up to 70% of the obtained monoliths were able to be used for anions separation. Nevertheless, the resulted separation efficiency was still not as good as those obtained by using particle-packed columns [13–15].

**Effect of eluent cation**

From our previous results [13], the eluent cations are expected to be coordinated among multiple PEG chains and work as the anion-exchange sites, therefore the effect of eluent cations was investigated and the separation results are shown in Figure 7. Figure 7 (A) was obtained using monovalent eluent cations while Figure 7 (B) was obtained using divalent eluent cations. The results showed that divalent cations could be trapped stronger than those monovalent cations, as the separation profiles of anions showed better peak shape and longer retention time. It seems that the size of eluent cations may not be very significant for coordinating the eluent cations. In addition, using acidic eluent condition showed better separation of the anions; the reason for such retention behavior is still under investigation.

**Effect of eluent concentration and separation mechanism**

The effect of eluent concentration, i.e. salt concentration as the eluent on the retention of anions was investigated using CaCl2 as the eluent, and the separation results are shown in Figure 8.

**Figure 7.** Separation of inorganic anions on PEG-monolithic columns F using (A) monovalent and (B) divalent eluent cation.

Column: PEG-monolith F (100 x 0.32 mm I.D.). Eluent: as indicated; 20 mM each + 1 mM HCl. Other operating conditions as in Figure 3.

**Figure 8.** Effect of eluent concentration on the retention of inorganic anions.

Column: PEG-monolith F (100 x 0.32 mm I.D.). Eluent: CaCl2 concentration as indicated. Other operating conditions as in Figure 3.
As found in common IC, the retention of anions decreased with increasing eluent concentration. It is also known that the plots of the logarithm of the retention factor (log k) of the anions as a function of the logarithm of the eluent concentration are linear. These plots of the anions were found to be almost linear (R² between 0.948-0.994), and the slopes of each line were calculated to be between -0.16 and -0.20.

Theoretically, if only ion-exchange mechanism was involved in the retention, the slopes should be -0.5 because the eluent cation is divalent; the obtained results of less than -0.2 showing that besides ion-exchange, another retention mechanism was also involved.

As shown in Figure 9 below, the eluent cations were trapped between the PEG chains via ion-dipole interaction with the oxygen atoms, and worked as the anion-exchange sites. The results showed that the eluent cations could be trapped by multiple PEG chains, and it is also expected that the analyte anions were also retained via partition mode. Further investigation is necessary to calculate the effect of these interactions in order to improve the separation efficiency.

![Figure 9. Illustration of the separation mechanism.](image)

**Figure 9.** Illustration of the separation mechanism.

**Repeatability and theoretical plates**

The relative standard deviations (RSDs) of retention time, peak height, and peak area for five chromatographic separations for the five anions were calculated to be between 1.50-3.17, 2.12-7.51, and 1.93-9.51%, when 20 mM CaCl₂ was used as the eluent. The theoretical plate numbers calculated for IO₃⁻, NO₃⁻ and NO₂⁻ were 3430, 4340, and 3900, respectively, while those of I⁻ and SCN⁻ were 710 and 1000 plates, respectively. The low plate numbers were caused by peak tailing, and this could be due to the solvation strength of the anions. Nevertheless, compared to our previous study [16], i.e. the highest plate number achieved was 3346 plates/m (calculated from the retention of nitrate), the current PEG-monolith clearly shows an improvement in terms of peak shape. However, the degree of resolution between peaks 1-3 (IO₃⁻, NO₃⁻ and I⁻) need further optimization.

**Conclusions**

A simple one-pot-single-step polymerization of PEG-monolithic columns was reported and the resulted monoliths showed comparable good separation efficiency for the five inorganic anions involved. The eluent cations were trapped among PEG chains and acted as the anion-exchange sites. Nevertheless, the ion-exchange mechanism was not the sole retention involved in the PEG-monoliths. The retention of NO₂⁻ was found to be the longest among the tested anions, showing the possibility of other separation mechanisms. Further investigation using different PEG monomers is still undergoing, and obtaining long-term stability still remains as a challenging task.

**Conflict of Interest**

The authors declare that there is no conflict of interest of whatsoever case.

**References**


