Introduction

In liquid chromatography, including ion chromatography, particle-packed columns filled with spherical silica gel or polymer particles having sizes of between 3 – 5 μm are generally employed.\(^1\)\(^-\)\(^4\) In a particle-packed column, when the particle diameter is reduced, a dedicated high pressure-resistant system is required because the load pressure applied to the column increases in inversely proportion to the square of the charged particle diameter. In recent years, monolithic columns have attracted attention as separation columns that only require low-pressure loads.

The term monolith refers to a porous body having three-dimensionally connected pores; in particular, it comprises a bulk body with a three-dimensional network structure having micrometer-sized pores (macropores) and nanometer-sized pores (mesopores). Therefore, the monolithic stationary phase has a high porosity as well as superior permeability in the mobile phase compared with the porosity of a particle-packed stationary phase column, permitting high-speed and high-performance separation with a relatively low pressure loss.

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The monolithic column is roughly classified according to the type of silica and organic polymer used in preparing it. A silica-based monolith is prepared by the sol-gel method using alkoxysilane, and it generally has a relatively high porosity of 80% or more.\(^5\)\(^-\)\(^13\) Furthermore, it displays a high mechanical strength and excellent separation performance. On the other hand, an organic polymer-type monolith is prepared by reacting a solution comprising a copolymerized monomer, a crosslinking agent, a pore-forming agent (“porogen”), and a polymerization initiator in the same type of column.\(^14\)\(^-\)\(^20\) The organic polymer-type monolithic column is easier to be prepared, and it displays excellent pH durability; as such, irreversible adsorption rarely occurs.

In ion chromatography, crown ethers, such as 18-crown-6-ether (18C6E), are added to the eluent to control the retention time of specifications.\(^21\)\(^-\)\(^24\) In capillary ion chromatography, a small amount of 18C6E is added to the eluent together with acetonitrile during the separation of inorganic anions. Thus, the 18C6E captures cations from the eluent and acts as an ion-exchange site.\(^25\),\(^26\) However, according to the revised 6th edition of the Globally Harmonized System of Classification and Label of Chemicals (GHS),\(^27\) regarding the classification and labeling of chemicals by the United Nations Economic Commission for Europe (UNECE), 18C6E is classified as a Category 4 compound for acute toxicity (oral) and a Category 2 compound concerning skin corrosiveness/irritation. Therefore, it is undesirable to discard 18C6E in waste solutions, even in trace amounts. This drawback could be overcome by changing the use of 18C6E as an eluent additive to, for example, a chemically bonded stationary phase in order to keep its specifications for use in ion chromatography.

Nevertheless, an example of using crown ether as the stationary phase in liquid chromatography is chiral analysis using a stationary phase coated or chemically bonded with optically active crown ether on the surface of silica gel. Examples of the optically active crown ether include (18-crown-6)-2,3,11,12-tetracarboxylic acid and benzo-18-crown-6-ether derivative. By ionizing (-NH\(^3\)\(^+\)) the amino group of a primary amine compound, such as an amino acid, the amino group is inscribed in a crown ether to enable chiral analysis.\(^28\),\(^29\)

Conversely, our research group has developed a facile chemical bonding reaction between the glycidyl group of 3-glycidoxypropyltrimethoxysilane (GPTMS) and the amino group

Separation of Inorganic Anions Using an 18-Crown-6-ether-modified Organic Polymer Monolithic Stationary Phase in Capillary Ion Chromatography

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In this study, a monolithic organic polymer stationary phase was modified using 18-crown-6-ether for use in capillary ion chromatography. Its use in the separation of inorganic anions was investigated. The monolithic stationary phase was obtained by chemically bonding 2-aminomethyl-18-crown-6-ether to a polymer skeleton comprising glycidyl methacrylate and ethylene glycol dimethacrylate. The optimum level of the loading of 2-aminomethyl-18-crown-6-ether onto the stationary phase was investigated. The resulting stationary phase was used to investigate the influence of the eluent cation, the concentration of the eluent, and the pH of the eluent on the separation of inorganic anions.

Keywords Crown ether-bonded stationary phase, inorganic anions, capillary ion chromatography

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of 2-aminomethyl-18-crown-6-ether (2AM18C6E) to analyze inorganic anions using a 18C6E chemical bonded stationary phase.\textsuperscript{30} In addition, by bonding 1-aza-15-crown-5-ether (1A15C5E), 1-aza-18-crown-6-ether (1A18C6), or 2AM18C6E to silica gel via GPTMS, the separation of anions and cations was achieved.\textsuperscript{31} However, since these stationary phases are based on silica materials, they have limited usage under basic environments.

These previous studies suggest that the chemical analysis of anions, cations and optically active substances can be realized with one analytical medium by chemically bonding an appropriate crown ether derivative to the stationary phase. On the other hand, in previous studies, the stationary phase was silica-gel particles, and there were restrictions on usable pH and pressure.

In this study, in order to obtain a separation media that can be used in basic regions, having higher porosity as well as easy preparation, an organic polymer-based monolith composed of glycidyl methacrylate (GMA) and ethylene glycol dimethacrylate (EGDMA) was synthesized in fused-silica capillaries. The monolith was then functionalized with 2AM18C6E and the retention behavior of several inorganic anions as well as the influence of the eluent pH on its performance were investigated.

### Experimental

#### Reagents and materials

All reagents were guaranteed-grade products and obtained from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), unless noted otherwise. 3-(Trimethoxysilyl) propyl methacrylate was obtained from Tokyo Chemical Industry Co., Ltd., (Tokyo, Japan), and 2AM18C6E was obtained from Sigma-Aldrich Co. LLC (St. Louis, USA). Sodium iodate, sodium bromate, sodium nitrate, sodium nitrite, ammonium chloride, and cesium chloride were obtained from Nacalai Tesque, Inc., (Kyoto, Japan). Calcium chloride, rubidium chloride, and lithium chloride were obtained from Yoneyama Yakuhin Kogyo Co., Ltd., (Osaka, Japan).

#### Apparatus

All experiments were performed using a capillary LC (liquid chromatography) system constructed from the following devices. The pump was a microfeeder (L.TEX Corporation, Tokyo, Japan) equipped with a 0.5 mL gas-tight syringe (ITO Corporation, Fuji, Japan). A capillary column prepared from a fused-silica capillary tube (100 × 0.32 mm i.d., GL Sciences Inc., Tokyo, Japan), an M-435 micro injection valve (Upchurch Scientific, Oak Harbor, Wash., USA) with injection volume of 0.2 μL, and a UV detector model UV-2075 (JASCO Corporation, Tokyo, Japan) used at a detection wavelength of 210 nm were used. CDS-Lite Ver. 5.0 (LAsoft LTD., Chiba, Japan) was used to process the data. The inlet pressure was monitored by a L.TEX-8150 pressure sensor (L.TEX Corporation). The separation columns were immersed in a water bath at 25 ± 1°C to maintain a stable temperature throughout the study.

An elemental analysis of the prepared stationary phase was conducted by using a Micro Corder JM10 (J-Science Lab Co., Ltd., Kyoto, Japan). FT-IR spectra of the monoliths before and after reacting with crown ether were taken using a Spectrum 400 FT-IR Spectrometer (PerkinElmer, Waltham, MA, USA) equipped with an all-reflective optics device GladiATR (PIKE Technologies, Madison, WI, USA). The pH was measured using an HM-41 type pH meter (electrode GST-5821C) manufactured by TOA DKK Corporation (Tokyo, Japan).

#### Preparation of stationary phase

The stationary phase was prepared using a methacrylic acid-based polymer that has been studied for a long time by our research group.\textsuperscript{32} The conditions for reacting the crown ether with the glycidyl group were in accordance with a report by our research group.\textsuperscript{30,31} Generally, one of the main challenges in making monolithic stationary phases is the column preparation repeatability. Therefore, crown ether bonded monolithic capillary columns were prepared (under optimized conditions) and evaluated repeatedly in this study.

1.0 M NaOH, 1.0 M HCl, and an acetone solution of 30% (v/v) 3-(trimethoxysilyl) propyl methacrylate was obtained from Tokyo Chemical Industry Co., Ltd., (Tokyo, Japan), and 2AM18C6E was obtained from Sigma-Aldrich Co. LLC (St. Louis, USA). Sodium isodate, sodium bromate, sodium nitrate, sodium nitrite, ammonium chloride, and cesium chloride were obtained from Nacalai Tesque, Inc., (Kyoto, Japan). Calcium chloride, rubidium chloride, and lithium chloride were obtained from Yoneyama Yakuhin Kogyo Co., Ltd., (Osaka, Japan).

The pretreatment steps were necessary to activate and attach methacrylate groups onto the inner wall of the silica capillary tube. The silica capillary tube was then washed with acetone and dried under a flow of nitrogen gas. Then, 0.225 mL of GMA monomer, 0.075 mL of EGDMA as the crosslinking agent, 0.4 mL of 1-propanol, 0.25 mL of 1,4-butanediol, 0.05 mL of H2O as porogens, and 1.0 mg of 2,2′-azobisobutyronitrile (AIBN) as the polymerization initiator were thoroughly mixed in an ultrasonic disperser and injected into the pretreated silica capillary tube using a microsyringe. The mixture was allowed to react in a water bath at 60°C for 24 h to prepare the glycidyl group containing organic polymer monolith in the fused-silica capillary tube. Next, a mixture of 0 - 25 mg 2AM18C6E,
0.228 mmol g–1. The 18C6E content was lower than that in a previous study by our research group (previously it was 0.393 mmol g–1). The following are possible reasons for this.

Effect of the 2AM18C6E/glycidyl ratio on the retention of the analyte anions. Column, 100 × 0.32 mm i.d.; eluent, 30 mM KCl; flow-rate, 2.0 μL min–1; wavelength of UV detection, 210 nm; analyte, 0.2 mM each (0.2 μL): 1, IO3–; 2, BrO3–; 3, NO2–; 4, NO3–.

Results and Discussion

Effect of the 2AM18C6E/glycidyl ratio

Stationary phases were prepared by optimizing the ratio of 2AM18C6E to glycidyl groups during preparation, i.e., the 2AM18C6E/glycidyl ratio (theoretical value) was varied from 0/100 to 25/100. Four types of anions, namely IO3–, BrO3–, NO2–, and NO3–, were separated using the modified stationary phases. The results are given in Fig. 2.

From a 2AM18C6E/glycidyl ratio of 0/100 to 15/100, the retention time increased with an increasing amount of 2AM18C6E, whereas, above 20/100 no obvious change in the retention time was observed. Theoretically, 2AM18C6E reacts with the glycidyl groups in a ratio of 1:1, but there is an upper limit to the amount of 2AM18C6E that can be chemically bound to the stationary phase. This limitation may be due to a steric hindrance between the 18C6E units, the presence of glycidyl groups that are not facing the cavity side of the monolith, and the porosity of the organic polymer monolith being smaller than that of the silica monolith. The 18C6E content in the stationary phase having a 2 AM18C6E/glycidyl ratio of 20/100, determined from the nitrogen content by elemental analysis, was 0.228 mmol g–1. The 18C6E content was lower than that in a previous study by our research group (previously it was 0.393 mmol g–1). The following are possible reasons for this.

Effect of the eluent concentration on the retention of analyte anions

By changing the concentration of KCl in the eluent from 10 to 220 mM, four anions, namely IO3–, BrO3–, NO2–, and NO3–, were separated. The analytical conditions and results are shown in Table 1.

Table 1 Repeatability of chromatograms, RSD, % (n = 6)

| Analyte pH of eluent | IO3– | BrO3– | NO2– | NO3– |
|----------------------|------|-------|------|------|
| Retention time       | 2.0  | 0.0   | 2.1  | 2.4  |
| Peak height          | 1.3  | 8.5   | 3.5  | 1.3  |
| Peak area            | 1.9  | 17.6  | 3.9  | 2.5  |

In a previous study by our research group, excess 2AM18C6E was added to epoxy silica gel and reacted in a beaker. In this study, a polymer monolith was prepared in a capillary tube in advance, and the column was filled with 2AM18C6E and reacted. Furthermore, in this study, it is considered that the mass of the stationary phase in the capillary tube is smaller than that of our previous study. In subsequent experiments, the 2AM18C6E/glycidyl ratio was set to 20/100 unless specified otherwise.

Confirmation of chemically bonding crown ether to the monolith

Generally, in the case of particulate stationary phases, elemental analysis is used to confirm and calculate the amount of reacted chemicals. However, for the case of monolithic capillary columns, it is almost impossible to perform elemental analysis. This is because a certain amount of a sample is required for the elemental analysis; however, it is difficult to obtain a sufficient amount of samples from a capillary column. In addition, when the monoliths are prepared in a column with a large inner diameter or in a vial, the pore properties may change compared to those of the monoliths prepared in a capillary.

Therefore, a chromatographic separation of the retained analytes was carried out to prove the presence of crown ether, and washing with an organic solvent (such as methanol and acetonitrile) could prove that the modification involved chemical bonding (rather than just a physical attachment). In addition, as straightforward evidence, the FT-IR spectra before and after the reaction with crown ether were also taken.

After measuring a sample containing four anions, the monolithic column was washed with 0.5 mL of methanol. Thereafter, it was used to perform the same separation; the results of these measurements are given in Fig. S1. Since no significant change was observed in the chromatogram before and after washing the column with methanol, it is considered that 2AM18C6E and the glycidyl group were chemically bonded. In addition, the FT-IR spectra before and after the modification with crown ether, as shown in Fig. S2, indicated a C=O–C bond at 1050 cm–1, and a C–H stretch as well as a N–H stretch at 2950 and 3680 cm–1, respectively, attributed to 2AM18C6E. Furthermore, as shown in Table 1, the repeatability in six repeated measurements also suggests that 2AM18C6E and the glycidyl group are chemically bonded.

The pH value of the eluent was measured using a pH meter. Eluent: 30 mM KCl (pH = 6.25), 30 mM KOH (pH = 12.31). Other operating conditions as in Fig. 2.

a. Could not be identified due to overlap with the large negative peak (please refer to Fig. 7).
Fig. 3. With increasing KCl concentration, the retention time became shorter and showed the same tendency as reported previously. However, the observed change in the retention time with increasing KCl concentration was moderate, probably because the K⁺ and Cl⁻ ions in the eluent act antagonistically. The following experiment was performed to investigate the interaction between K⁺ ions and Cl⁻ ions. Four kinds of anions were analyzed using K₂SO₄ as the eluent and changing the K₂SO₄ concentration (Fig. 4). It is considered that by increasing the K⁺ ion concentration in the eluent, the anion retention is stronger. However, when the K₂SO₄ concentration ranged from 0.5 to 2 mM, the retention tended to decrease. The cause of this is still under investigation. In addition, while keeping the KCl concentration in the eluate constant, LiCl was added to the eluate to analyze four kinds of anions (Fig. 5). As a result, there was a tendency that the retention was shortened when the LiCl concentration in the eluent was increased. This is because the Cl⁻ ion concentration in the eluent was higher, resulting in an increase in the elution strength. In other words, the retention of anions in the sample can be controlled by the potassium ion concentration and the chloride ion concentration.

Effect of the eluent cation on the retention of target anions

The eluent cation was changed to study the separation of the four anions, namely IO₃⁻, BrO₃⁻, NO₂⁻, and NO₃⁻. The experimental conditions and results are given in Fig. 6. As previously reported by our group, the retention tended to be strong when the stability constant (Table S1) between 18C6E and the eluent cation was large. Therefore, the retention of anions in the sample can be controlled by the type of cation in the eluent.
Effect of pH of the eluent and repeatability of chromatogram

IO₃⁻, BrO₃⁻, NO₂⁻, and NO₃⁻ were separated by changing the pH of the eluent from 6 to 13. The experimental conditions and results are given in Fig. 7 and Table 1. Although the retention time varied depending on the pH of the eluent, separation could be achieved at any pH. At pH = 12.3, the BrO₃⁻ ion could not be detected due to a large negative peak (Fig. 7). Therefore, the stationary phase based on the organic polymer monolith prepared in this study proved to be applicable over a wide range of pH, which is an improvement on a silica based stationary phase that has limited usage in a basic environment. The Japanese Industrial Standard “JIS K 0102 Testing Methods for Industrial Wastewater” has the following description. If it is not possible to measure immediately after sampling, the samples used for measuring the iodide ion and the bromide ion should be stored at approximately pH = 10 with a sodium hydroxide solution. Samples used for cyanide and sulfide ion measurements should be stored at approximately pH = 12 with sodium hydroxide solution. Since this stationary phase can be used even in the basic region, it may be possible to use it for the analysis of these samples in the future.

Conclusions

This study revealed that the 18C6E chemically bonded organic polymer monolith stationary phase captures eluent cations and enables the separation of inorganic anions. The nature of the eluent cation considerably affects the retention of the analyte anions. It was also suggested that retention of analyte anions could be controlled by the eluent anions and cations. Furthermore, using an organic polymer monolith for the stationary phase, anions could be separated even in a basic region.

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Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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