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## Separation of Inorganic Anions and Phenolic Compounds Using Tetraethylene Oxide-Bonded Stationary Phases in Capillary Liquid Chromatography

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**Abstract:** Tetraethylene glycol monomethyl ether (TEGMM) and tetraethylene glycol (TEG) modified silica were synthesized and used in capillary liquid chromatography as stationary phases. The stationary phases were prepared by chemically bonding TEGMM or TEG on silica via reaction with 3-glycidyloxypropyltrimethoxysilane. The present stationary phases were successfully used for the separation of several inorganic anions and phenolic compounds. The TEG-modified silica stationary phase had a better selectivity and higher retention for five anions compared to TEGMM-modified silica stationary phase. The repeatability of retention time for the five anions was satisfactory on both stationary phases. By using 2 mM sodium chloride as eluent, the relative standard deviation values were in ranging from 0.68–3.21 and 2.00–2.16% for analytes in the TEG- and TEGMM-bonded stationary phase, respectively. It was found that the TEG-bonded stationary phase had hydrophilic properties due to the presence of the hydroxyl group at the end of the TEG chains.

**Keywords:** capillary liquid chromatography; tetraethylene glycol monomethyl ether; tetraethylene glycol; inorganic anions; phenol compounds

### ■ INTRODUCTION

Liquid chromatography (LC) has many advantages in the separation and analysis of complex samples because LC has many choices in the stationary phases with excellent performance. Excellent performance in the chromatographic techniques is directly influenced by the separation mechanism of the stationary phase. In the traditional stationary phases, the primary separation mechanism is often single, such as hydrophobic interaction for separation of nonpolar compounds on reversed-phase mode and ion-exchange interaction for separation of ions on ion-exchange mode [1].

Ion chromatography (IC) was introduced in 1975 and after that many types of stationary phases and separation modes have been developed in IC [2]. Silica-based stationary phases are the most developed stationary

phases in IC by many researchers because silica has several advantages [3-10]. Some of the benefits using silica are resistance to organic solvents, high rigidity, fast mass transfer, and easy introduction of many functional groups because of rich in silanol groups on the surface of silica. But, silica also has some disadvantages such as could not be used in strongly acidic or alkaline mobile phases, and residual silanols affect the separation of biological samples. An example of modifying silica into new stationary phases is 4,4'-dipyridine-modified silica as multi-interaction stationary phase synthesized by Sun et al. [11]. By using this new stationary phases, they could separate the inorganic and organic anions individually by anion-exchange mode, while polycyclic aromatic hydrocarbons and phenolic compounds were successfully separated in the reversed-phase mode using the same stationary phase [11].

In IC, separation of ions is based on the differences in the electrostatic attraction or repulsion between the analyte and the stationary phase. So, the stationary phase used for IC usually has a charged or chargeable moiety in their functional group. Besides using ion exchange mode, inorganic anions could also be separated in the stationary phase with no ion exchange site. A crown ether- and cryptand-bonded stationary phase have been developed by Lamb and Smith for separation of alkali metal cations as well as anions [12-13]. Takeuchi et al. [14-15] have been developed a polyoxyethyleneonaminopropyl silica stationary phase for the determination of inorganic anions in saliva. This stationary phase expected to retain anions in the ion exchange mode because of cations from the eluent is coordinated among the multiple poly(ethylene oxide) (PEO) chains via ion-dipole interaction, working as the anion exchange sites. Three types of crown ethers (i.e., 1-aza-15-crown-5-ether, 1-aza-18-crown-6-ether and 2-aminomethyl-18-crown-6-ether) chemically bonded to silica gels via 2-step reaction also developed by Takeuchi et al. for the separation of both anions and cations [8,16].

From our previous study, a tosylated-poly(ethylene oxide) was used as PEGylation reagent for the preparation of PEO-bonded stationary phase to separate inorganic anions in capillary IC [7]. In this paper which is part of the first author Ph.D. theses [17], a shorter chain of ethylene oxide, i.e., tetraethylene glycol monomethyl ether- and tetraethylene glycol-modified silica was used as a new stationary phase for IC. These reagents were chosen because they are commercially available and not so expensive compared with the previous reagents. In addition, these new stationary phases were expected to give the same separation profile. The separation of inorganic anions and polar compounds such as phenolic compounds using prepared stationary phases was evaluated as well.

## ■ EXPERIMENTAL SECTION

### Materials

Pyrocatechol, tetraethylene glycol (TEG), toluene, and uracil were obtained from NacalaiTesque (Kyoto, Japan). Tetraethylene glycol monomethyl ether

(TEGMM) and 3-glycidyloxypropyltrimethoxysilane (GPTMS) were purchased from Tokyo Chemical Industry (Tokyo, Japan), phenol and pyrogallol were obtained from Wako Pure Chemical Industries (Osaka, Japan). Other reagents and solvents used in the experiments were analytical grade and used without further purification. Purified water was produced in the laboratory using a GS-590 water distillation system (Advantec, Tokyo, Japan) and used in this work for the preparation of inorganic sample solutions and eluents. Before use, toluene was dried over 3A (1/16" pellets) molecular sieves. In this study, silica gel with an average particle diameter about 5  $\mu\text{m}$  with a mean pore diameter of 12 nm (Chemical Evaluation and Research Institute, Japan) was used and dried at 110  $^{\circ}\text{C}$  for 4 h before use. A stainless-steel tubing with 250  $\times$  4.6 mm i.d. was used for the reaction vessel

### Instrumentation

A  $\mu\text{LC}$  system was used for the chromatographic separation. The eluent was supplied by L.TEX-8301 Micro Feeder (L. TEX Corporation, Tokyo, Japan) pump equipped with MS-GAN 050 gas-tight syringe (0.5 mL; Ito, Fuji, Japan) at a flow rate of 3  $\mu\text{L min}^{-1}$ . The inlet pressure was monitored by an L.TEX-8150 pressure sensor (L.TEX). A microinjection valve model M435 (Upchurch Scientific, Oak Harbor, WA, USA) with an injection volume of 0.2  $\mu\text{L}$  was used as the injector. A UV-970 UV-Vis detector (Jasco, Tokyo, Japan) was operated at 210 or 254 nm. CDS data processor (LASOFT, Chiba, Japan) was used for data collection. The synthesized packing materials were evaluated for its elemental composition using MT-6 CHN Corder (Yanaco, Kyoto, Japan).

### Procedure

#### **Preparation of TEGMM-bonded and TEG-bonded stationary phases**

About 0.2 g dried porous silica gel, 3.5 mL dry toluene, and 0.2 mL GPTMS were added into a 20 mL vial, and the mixture solution was then poured into a stainless steel tubing. The reaction was carried out at 110  $^{\circ}\text{C}$  for 20 h with the vessel was occasionally vibrated manually during the reaction. The reacted silica gel as

the result of the reaction was washed with dry toluene followed by drying at 75 °C for 4 h. To the dried reacted silica gel, 3.5 mL N,N-dimethylformamide (DMF) was added followed by adding 0.2 mL TEG or TEGMM and then poured into the reaction vessel. The reaction was conducted in an oven at 120 °C for 24 h for TEG and at 75 °C for 24 h for TEGMM, followed by washing with methanol. The expected scheme of the reaction as shown in Fig. 1. The separation column was prepared from a fused silica capillary tube (0.32 mm i.d. × 100 mm) using a slurry packing method reported previously [6].

## RESULTS AND DISCUSSION

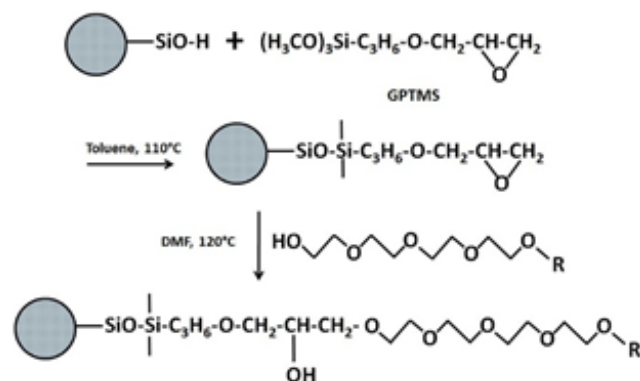
### Elemental Analysis of the Stationary Phases

The Synthesized TEGMM- and TEG-bonded stationary phases were characterized by elemental analysis. The percentages of carbon and hydrogen contained in the Si-GPTMS-TEGMM were 7.87 and 1.72% (w/w), while the percentages for the Si-GPTMS-TEG were 7.45 and 1.64% (w/w), respectively. According to the results, carbon and hydrogen contained in the Si-GPTMS-TEGMM were 6.6 and 17.1 mmol/g, while carbon and hydrogen contained in the Si-GPTMS-TEG were 6.2 and 16.3 mmol/g, respectively.

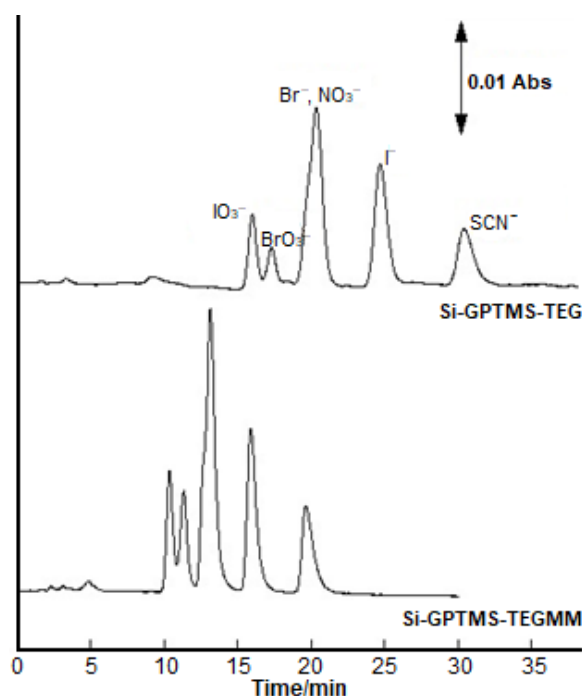
### Retention Behaviors of Anions on TEGMM-Bonded and TEG-Bonded Stationary Phase

Separation of six anions in both prepared stationary phases could only retain five anions satisfactorily in a reasonable time. Bromide and nitrate are not entirely separated in the two stationary phases. From the previous work by using a longer chain of ethylene oxide, i.e., PEO-bonded silica, it was concluded that ion-dipole interaction occurred from the coordination of the cations of eluent with the oxygen atoms of multiple PEO chains, which then worked as the anion exchange sites. So, the eluent and analyte anions competed for the trapped cations [14]. The elution order of the six inorganic anions observed was similar to that found in typical IC, which was iodate, bromate, bromide and nitrate, iodide, and thiocyanate.

Fig. 2 showed the chromatogram of the separation of six anions in the TEG- and TEGMM-bonded silica stationary phase with 2 mM sodium chloride as the mobile



**Fig 1.** The scheme of the expected reactions. TEGMM, R=CH<sub>3</sub>; TEG, R=H



**Fig 2.** Separation of six anions on TEG- and TEGMM-bonded silica stationary phase. Column, (100 × 0.32 mm i.d.); eluent, 2 mM sodium chloride; flow-rate, 3.0 μL/min; analytes, iodate, bromate, bromide, nitrate, iodide, thiocyanate; concentration of analytes, 0.1 mM each; injection volume, 0.2 μL; wavelength of UV detection, 210 nm

phase. The two stationary phases give slightly in the separation profile of the six anions. It can be seen that TEG-bonded silica gave better selectivity and stronger retention for the six anions compared to the TEGMM-bonded silica stationary phase. Different in the selectivity and retention of the analytes may be due to

the contribution of the hydroxyl group at the end of TEG chains in the coordination of eluent cations. Under the operating conditions are shown in Fig. 2, the repeatability of the retention time was evaluated and found the relative standard deviations of the retention time of the six anions were 0.68–3.21 and 2.00–2.16% for separation on TEG- and TEGMM-bonded silica stationary phase, respectively.

In this work, the reaction time and the temperature for the reaction TEG and TEGMM with silica were also optimized to obtain stationary phase with a better performance. The reaction time, and the reaction temperature were varied from 24 to 72 h, and 50 to 120 °C, respectively. From the result obtained, the reaction time for the chemically bonding of the TEG and TEGMM gave influences in the retention time of anions. Increasing the reaction time will decrease the retention time of anions in the two stationary phases. It seems that a longer reaction time causes the unraveling of PEO which have been bonded to the silica gel.

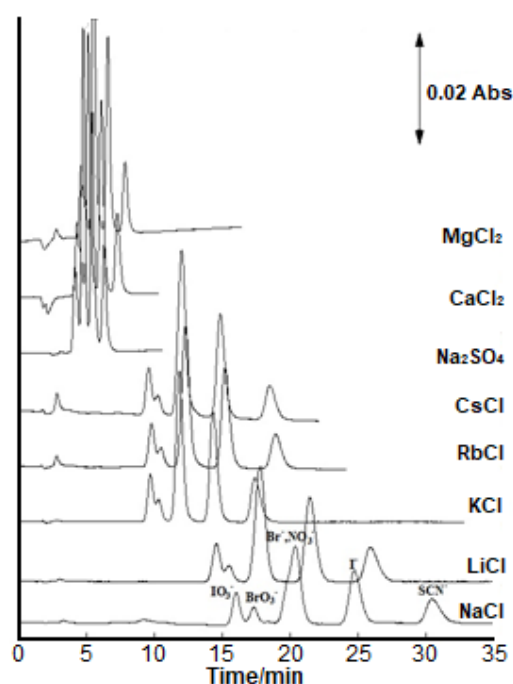
The reaction temperature of chemical bonding gave a different result for the two prepared stationary phases. In the TEG-bonded stationary phase, increasing temperature caused an increase in selectivity and stronger retention for anions. On the other hand, increasing temperature slightly decreased the selectivity and retention of anions on the TEGMM-bonded stationary phase. In this study, 24 h was chosen as the optimum reaction time for the preparation of both ethylene oxide-bonded stationary phases. In addition, the reaction temperature at 120 and 75 °C was chosen for the TEG-bonded stationary phase and the TEGMM-bonded stationary phase, respectively.

### Effect of Eluent Cation

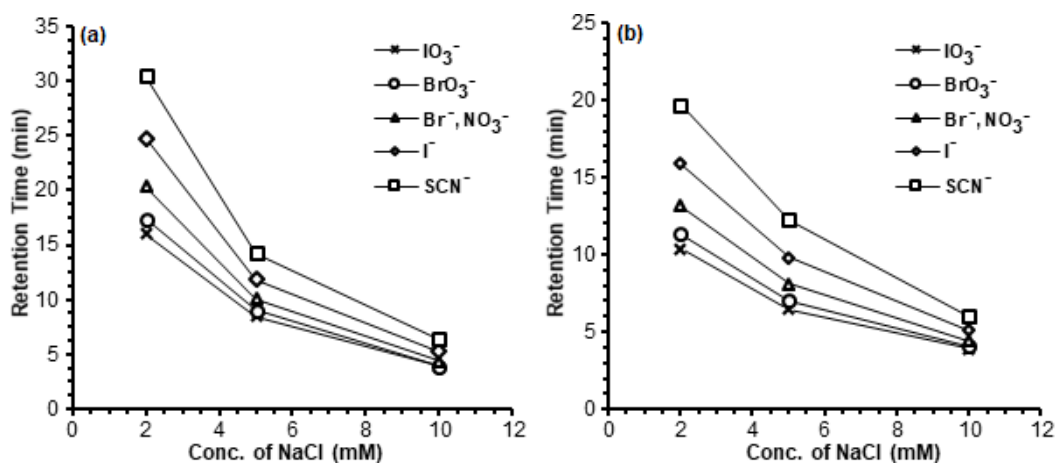
The retention of anions will be affected by the eluent cations on the prepared stationary phase because they are expected to coordinate among the multiple ethylene oxide chains and work as the anion exchange sites [17]. In addition, with a different eluent cation, a distinct retention behavior of analytes was observed. From our previous work with the PEO- bonded stationary phase, it has been showed that the size of the cations might not be

very significant for coordinating the eluent cations [7]. In this study, the longest retention time was achieved by using sodium chloride solution for the mobile phase. Separation of six anions on TEG-bonded stationary phase using 10 mM aqueous solution of various chlorides is shown in Fig. 3 with the elution order of all analytes with different eluents used was the same.

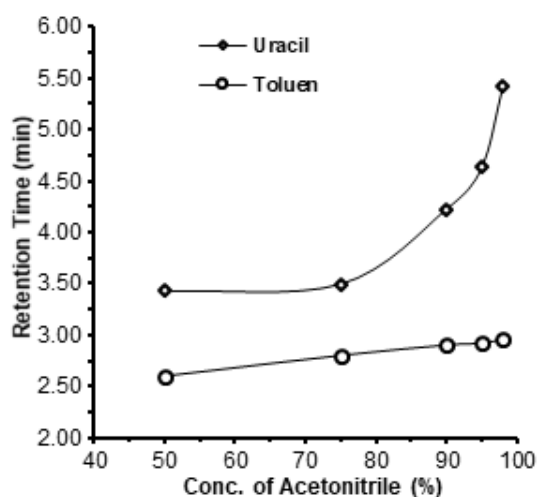
Among the eluents tested, sodium chloride gave the highest resolution, and the separation was achieved in a reasonable time. Thus sodium chloride was used as the eluent in the following experiments. Different concentration of sodium chloride was evaluated for examining the effect of mobile phase concentration on the retention of the six anions. Fig. 4 showed the retention time of the six analytes on the two stationary phase decreased with increasing the mobile phase concentration as found in common IC.



**Fig 3.** Separation of six anions on TEG-bonded stationary phase using 10 mM aqueous solutions of various chlorides and sodium sulfate. TEG-bonded silica (100 × 0.32 mm i.d.); eluents, as indicated; flow-rate, 3.0 µL/min; analytes, iodate, bromate, bromide, nitrate, iodide, thiocyanate; concentration of analytes, 0.5 mM each; injection volume, 0.2 µL; wavelength of UV detection, 210 nm



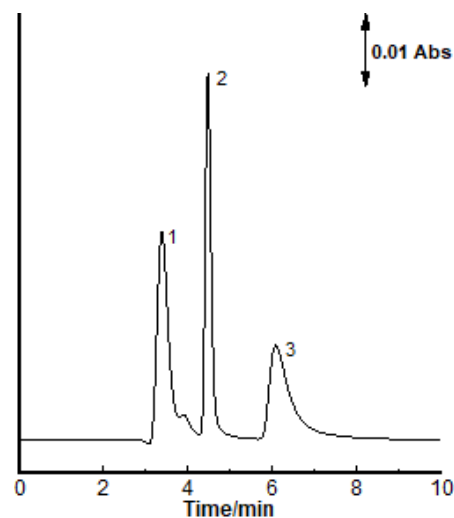
**Fig 4.** The retention time of six anions as a function of eluent concentration; (a) on TEG bonded stationary phase; (b) TEGMM bonded stationary phase. Operating conditions as in Fig. 2 except for the eluent as indicated



**Fig 5.** Retention times of uracil and toluene as a function of acetonitrile concentration in the mobile phase. Column, TEG bonded stationary phase (100 × 0.32 mm i.d.); mobile phase, acetonitrile-water mixtures; flow rate, 3  $\mu$ L/min; wavelength of UV detection, 254 nm; analytes, 0.002% (w/v) each of uracil and toluene; injection volume, 0.2  $\mu$ L

### The HILIC Mode Properties of TEG-Bonded Stationary Phase

Specific interaction such as dipole-dipole and hydrogen bonding is expected to play in the separation of polar compounds in the TEG-bonded stationary phase since it has a hydroxyl group in its chains. In this paper, the properties of hydrophilic interaction liquid chromatography (HILIC) mode in the TEG-bonded stationary phase was investigated. Uracil and toluene

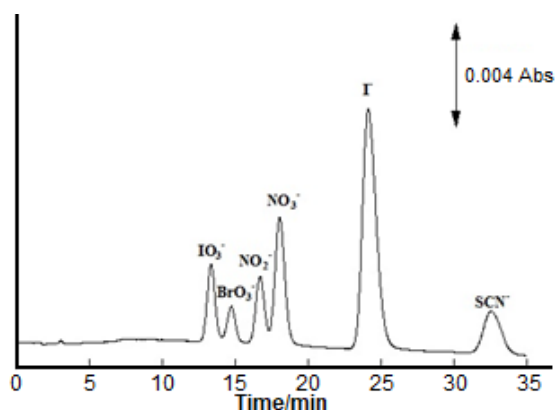


**Fig 6.** Separation of phenolic compounds. Column, TEG-bonded stationary phase (100 × 0.32 mm i.d.); mobile phase acetonitrile:water (98:2); flow rate, 3  $\mu$ L/min; wavelength of UV detection, 254 nm; analytes, 0.01% (w/v) each of phenol (1), pyrocatechol (2) and pyrogallol (3); injection volume, 0.2  $\mu$ L

were used as the analyte and acetonitrile from 50 to 98% (v/v) were used as the mobile phase. The retention time of uracil, which is a polar compound, increased when the concentration of acetonitrile risen from 50 to 98%, whereas the retention time of toluene, a nonpolar compound, was not influenced by increasing concentration of acetonitrile.

Separation of polar compounds in the TEG-bonded stationary phase was performed to evaluate the





**Fig 7.** Separation of six anions on TEG-bonded silica stationary phase which was prepared from 0.5 mL TEG added in chemical bonding. Column, (100 × 0.32 mm i.d.); eluent, 2 mM sodium chloride; flow-rate, 3.0  $\mu$ L/min; analytes, iodate, bromate, nitrite, nitrate, iodide, thiocyanate; concentration of analytes, 0.1 mM each; injection volume, 0.2  $\mu$ L; wavelength of UV detection, 210 nm

capability of the stationary phase for separating the three phenolic compounds, i.e., phenol, pyrocatechol, and pyrogallol. As can be seen in Fig. 6, the three analytes completely separated under an isocratic elution with analysis time less than 7 min.

#### Effect of the Amount of TEG

The amount of TEG was also varied from 0.2 to 1.0 mL to get better performance for the stationary phase. The retention times of the anions increased as a result of the increase in TEG volume from 0.2 to 0.5 mL. However, when the amount of TEG was 1.0 mL, the retention time of the anions decreased. The TEG-bonded silica stationary phase which was prepared from 0.5 mL TEG gave a different performance in the separation of anions compared to TEG-bonded silica stationary phase which was made from 0.2 mL TEG. By using 0.5 mL of TEG in the reaction, the TEG-bonded silica stationary phase could separate and determine nitrite and nitrate, whereas by using 0.2 mL of TEG, bromide, nitrite, and nitrate could not be separated (Fig. 7). The TEG-bonded silica stationary phase prepared from 0.5 mL TEG was used for application to a real sample.

#### Application to a Real Samples

The six anions and some phenolics compound could be separated with good selectivity and resolution in our TEG-bonded silica stationary phase. The TEG-bonded silica stationary phase was also used to determine the inorganic anions in saliva and tap water samples. It was found that nitrate in the saliva sample, and in the tap water was about 2.1, and 2.2 mg/L, respectively. Meanwhile, the other anions were not detected in both samples.

#### CONCLUSION

Tetraethylene oxide-bonded stationary phases by chemical bonding of TEGMM and TEG on silica via reaction with 3-glycidyoxypropyltrimethoxy-silane has been prepared. The TEG-bonded stationary phase had a better selectivity and higher retention for the five anions compared to the TEGMM-bonded stationary phase. In addition, the TEG-bonded stationary phase had hydrophilic properties. The present stationary phases were successfully used as stationary phases for the separation of inorganic anions and phenolics compounds.

#### ACKNOWLEDGMENTS

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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