Development of eluent-induced and monolithic stationary phases for separation of inorganic cations in capillary liquid chromatography

(キャピラリー液体クロマトグラフィーにおける無機陽イオン分離のための溶離液誘導型およびモノリス型固定相の開発)

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A dissertation submitted to the Gifu University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Material Engineering

By

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Short contents

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Summary

Analytical chemistry has become a basic technology in order to support the whole of sciences such as life science, environmental science and material science. In 1903, a Russian botanist Mikhail S. Tswett invented chromatography, and now chromatography has grown to be a powerful tool of analytical technique for separating, identifying, determining complex mixtures and also solves complicated problems of the analysis of environmental samples, biological fluids, oil and petroleum product, medicinal substances and many others.

Various chromatography methods have been developed to overcome the analytic problem. Liquid chromatography is more utilized than the other method such as gas chromatography because the sample analysed does not need to be vaporized. Other advantages of liquid chromatography are simple, rapid, sensitive, selective in determination*, etc*.

Recently, downsizing of column in liquid chromatography has become a trend and has attracted a great attention, because it can increase the mass sensitivity, can use the limited sample of volume, and it is environmentally friendly. It is also important to keep the performance of liquid chromatography in spite of miniaturization, although consumption of solvents, packing materials and reagents can be reduced by this system. In this dissertation we have developed an eluent-induced method and monolithic stationary phases for the separation of inorganic cations in capillary liquid chromatography.

Some of the eluent-induced methods have been introduced for ionic separation such as ion-pair chromatography, ion-interaction chromatography, soap chromatography, *etc*.

Perfluoroalkanesulfonic acids were selected as eluent additive because they are commercially available in the acid form. The eluent contained perfluoroalkanesulfonic acid, copper sulfate and methanol, where perfluoroalkanesulfonic acid was dynamically coated on the C30 stationary phase, while copper sulfate allowed indirect photometric detection of analyte cations. The methanol concentration was sensitive to the elution time of the analyte cations.

A non-suppressed contactless conductivity detector has been used as a capillary detector in a capillary ion chromatograph, combining a reversed-phase C30 column permanently modified with ionic surfactant. The C30 column (100 × 0.32 mm. i.d.) was modified with sodium dodecyl sulfate (SDS), an anionic surfactant, for the separation of inorganic cations. Methanesulfonic acid and SDS were employed as the mobile phase component, when the mixture of methanesulfonic acid and SDS was used as the eluent, the retention of cations was improved and baseline-separation of the cations was achieved within 23 min. The effect of the eluent composition on the retention behavior of inorganic cations was investigated. The repeatability of retention time and peak height were varied from 0.59 to 1.52 and 2.21 to 3.25% as relative standard deviation, respectively.

Since two decades ago, the innovations of many gifted analysts have brought us to a new era phase in chromatography, *i.e.*, separation using monolithic materials, both silica- or polymer-based. These materials have high permeability, no need to frits preparation and fast mass transfer. Polymer monolithic columns were used for separation of inorganic cations by using contactless conductivity detector. Glycidyl methacrylate (GMA) and polyethylene glycol dimethacrylate (PEGDMA) were used as monomer and crosslinker. Methanol and decanol were used as binary porogenic solvents. The monolith columns were prepared by two-step procedures: polymerization and sulfonation of GMA.

After we tried for separating inorganic cations by using poly (GMA/PEGDMA) prepared by 2 steps, we are interested in preparation by one pot synthesis of 2-acrylamido 2-methylpropane sulfonic acid (AMPSA) for separation of inorganic cations, because it is more simple preparation and AMPSA can be strong cation-exchange sites with sulfonic acid functional groups. AMPSA and EDMA were used as monomer and crosslinker. 1-Propanol, 1,4-butanediol and methanol were used as porogenic solvents. Although the polymer monolithic columns were employed for separation of inorganic cations, unfortunately this stationary phase only could separate Li^+, NH_4^+, Rb^+ and Ca^{2+}.

Chapter 1

Introduction

1.1 Principle of chromatography

Chromatography is a separation technique based on differences in the pattern of movement of molecules between the mobile phase and the stationary phase to separate the components (molecules) which are in solution. A Russian botanist Mikhail Semenovich Tswett (1872-1919) invented chromatography in 1903 when he was doing the research about plant pigments. He separated chlorophylls and carotenoids by using calcium carbonate, alumina and sucrose on a packed columns and petroleum ether/ethanol mixtures as eluents. The word chromatography defined from Greek words chroma (color) and graphein (to write) [1-3]. In 1906, Tswett published his two papers in Germany botanical journal with entitled: "Physical-chemical studies of chlorophyll adsorptions" [1] and " Adsorption analysis and chromatographic method. Application to the chemistry of chlorophyll [2].

Tswett gave the first definition of chromatography which is a method in which the components of a mixture are separated on an adsorbent column in a flowing system [1]. Another recommended definition from the International Union of Pure and Applied Chemistry (IUPAC) defines chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction [4].

Basically, chromatography is a separation method in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase), and the other a mobile phase. The mobile phase flows along the stationary phase. Stationary phase tends to hold the mixture components, while the mobile phase tends to carry them away. Based on the solubility of the mixture components can be separated, components that are less soluble in the mobile phase or stronger adsorbed on the stationary phase will be retained, while the more soluble components or less adsorbed will move faster.

There are two principle types of chromatography based on the mobile phase, they are gas chromatography (GC), in which the mobile phase is gas and liquid chromatography (LC), in which the mobile phase is liquid. The classification of chromatography techniques is shown in **Table 1-1** [5].

1.2 Capillary liquid chromatography

Capillary columns were originally invented for GC, but since 85% of known compounds are neither volatile not stable to be separated by GC, a novel technique for separation of non volatile compounds is very much desired and indispensable. In 1967, Horvath *et. al* [6] firstly introduced this technique to separate nucleotides by using capillary tubes instead of GC application into LC as well as small bore columns packed with glass beads in their fast-LC system. Since the invention of capillary LC, many types of columns have been developed.

Table 1-2 shows the development history of capillary-based separation methods and their related techniques. Ishii and co-workers used the word “micro” with LC, *i.e* microcolumn LC (μLC). The earlier stage of development of μLC was focused on achieving the higher resolution. For the case of densely packed columns, higher resolution could be achieved by

Table 1-1 Classification of chromatography

Mobile phase	Stationary phase	Name of chromatography	Abbreviation
1. Gas	Solid	Gas chromatography	GC
	Liquid		
2. Liquid	Solid	Liquid chromatography	LC
	Liquid		
3. Supercritical fluid		Supercritical fluid chromatography	SFC
Types of interactions			
1. Adsorption		Adsorption chromatography	-
2. Partition		Partition chromatography	-
3. Ion exchange		Ion-exchange chromatography	IC
4. Size-exclusion		Size-exclusion chromatography	SEC
Permeation		Gel permeation chromatography	GPC
Filtration		Gel filtration chromatography	GFC
5. Response to physical stimulations such as temperature, pH, etc		Responsive chromatography	-
Shape of separationbed			
1. Tubular		Column chromatography	-
2. Planar		Thin-layer chromatography	TLC
		Paper chromatography	PC

using smaller sizes of the packing materials, but the pressure drop across the columns was a delimitating parameter in the most cases. Therefore, open tubular columns were develop and they perfomed much superior permeability. The open-tubular columns refer to the columns with a retentive film or layer coated on the inside wall of the column tubing of which the diameter should be as small as possible. There are some advantages of capillary LC such as improved mass sensitivity due to small volume detection, lower consumption of stationary and mobile phase, so it enables us to use expensive chemical reagents and sample especially biological substances. With this condition this system can be quite friendly with the environment [7, 8].

Table 1-2 Development of capillary-based separation methods and their related techniques

Year	Separation methods and their related techniques
1974	Micro-HPLC
1978	Open-tubular capillary LC
1978	Packed microcapillary LC
1979	Fused-silica capillary
1981	Capillary zone electrophoresis
1985	Electrokinetic chromatography
1987	Capillary electrochromatography
1998	Monolithic silica capillary column

LC can be classified based on the column diameter such as preparative LC, conventional LC, semi-micro-LC, micro LC, and nano-LC [8], are shown in **Table 1-3.**

Table 1-3 Classification of separation columns in LC

Purposes	Classification	Inner diameter / mm	Flow rate / mL min^{-1}
Analytical	Nano-LC	< 0.075	< 0.00027
	Micro-LC	0.2 - 0.8	0.002 - 0.030
	Semi-micro-LC	1.0 - 2.1	0.047 - 0.21
	Conventional-LC	4.0 - 6.0	0.76 - 1.7
Preparative	Preparative LC	> 10	> 4.7

1.3 Polymer monolithic stationary phase in liquid chromatography

The definition of monolith come from Greek word, it means “one stone” or “one piece”. Monolith is a rigid macro-porous stationary phase, fabricated from either silica or polymer. In 1992, Svec groups firstly, produced the rigid macro-porous polymeric monolith column for chromatography [9]. Monolithic columns for LC were acquainted since two decades ago and have been applied in the most of LC separation modes. There are some advantages of monolith columns over packed columns such as use of the higher flow rate without a severe loss in efficiency, no need for frit to hold the phase, easiness to fabricate the monolith materials from liquid precursors in miniaturized columns format [10].

There are two types of monolithic columns based on their components: organic and inorganic polymers. Silica based monolith belongs to inorganic polymers. Soga and Nakanishi produced monolithic silica rods for chromatographic application [11-13]. Organic polymer monolithic materials were produced by in situ polymerization which contains monomers, cross-linker, porogens and silica monolithic columns are produced by so-gel process. Silica monoliths have

bimodal pores structures: the macro-pores and the meso-pores, with the result that silica monolith can separate the small molecules, but in some cases the proper use of meso-pores can get the good separation of large molecules (protein) [14]. On the other hand, polymer monolith material only has macro-porous structure which can use for separation of large molecules, because of its pore diffusion. Silica monoliths only can stable in the pH from 2-8, because silica can dissolve under alkaline conditions, but polymer can be stable in all pH range [15].

Figure 1-1 shows the structure of a typical rigid monolithic polymer (longitudinal section of a monolithic column) [16].

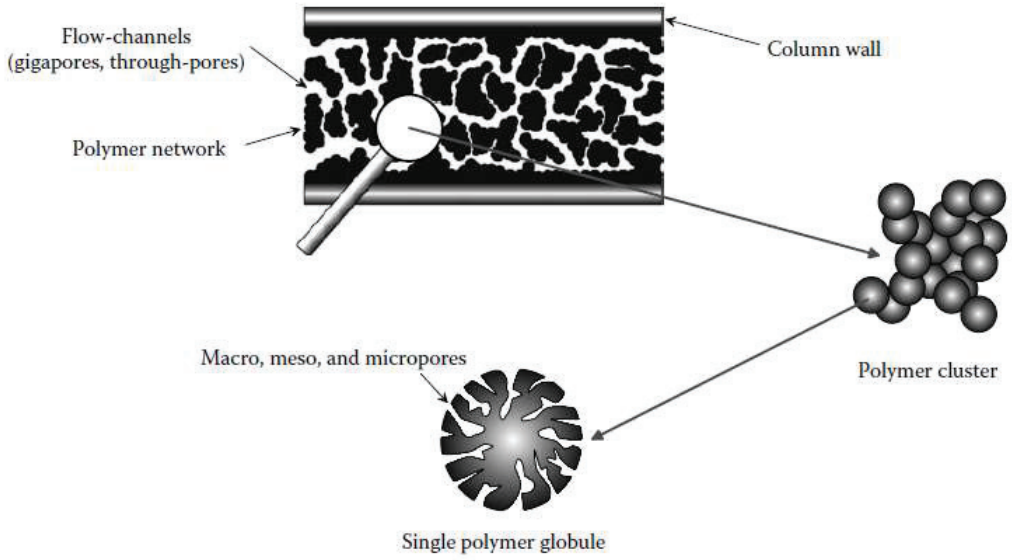


Fig. 1-1 Schematic representation of the structure and the morphology of a typical monolithic polymer prepared in HPLC column

The preparation of polymer monolithic column is easy and simple, firstly, polymerization of the mixture of monomer, crosslinker, porogenic solvent(one or more solvents), an initiator

(AIBN), is placed in capillary column and sealed on both ends. The polymerization is heated in the water bath at temperature 55^{o}C - 80^{o}C. Subsequently, the column is washed with the suitable solvent in order to remove the unreacted residues. Generally, organic polymer monolith can be classified into three general categories: polystyrene, methacrylate and acrylamide [15].

1.4 Ion chromatography

Ion chromatography is a subdivision of HPLC. The IUPAC gave the definition of ion chromatography :

"In ion exchange chromatography separation is based on differences in the ion exchange affinities of the individual analytes. If inorganic ions are separated and can be detected by conductivity detectors or by indirect UV detection then this is also called ion chromatography". For several reasons, the definition is not encouraging choice because it is only for the inorganic ions. It becomes difficult to understand if the system can separate the organic ions [17, 18]. Another definition as more general definitions: *Ion chromatography includes all rapid liquid chromatography separation of ions in columns coupled online with detection and quantification in a flow-through detector* [20].

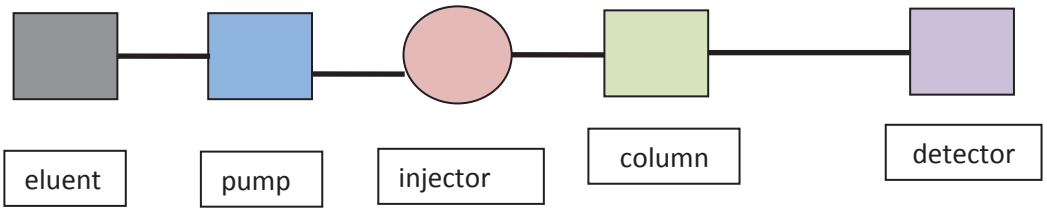
The term of ion chromatography was initiated by Small *et al.* in 1975 with the introduction of conductivity detection combined with the chemical reaction in conductivity. The history of ion exchange and ion chromatography as well as the technique of analytical based on ion exchange are briefly summarized in the table 1-4 [21].

Table 1-4 History of Ion Chromatography

ca. 1850	Soil as an ion exchanger for Mg^{2+}, Ca^{2+} and NH_4^+	Thomson & Way	**LC**
1935	Sulfonated and aminated condensation polymers (phenol/formaldehyde)	Adams, Holmes	
1942	Sulfonated PS/DVB resin as cation exchanger (Manhattan Project)	d'Alelio	
1947	Aminated PS/DVB resin as anion exchanger	McBurney	
1953	Ion exclusion chromatography	Wheaton, Baumann	
1957	Macroporous ion exchangers	Corte, Meyer, Kunin *et al.*	
1959	Basic theoretical principles	Helfferich	
1967-70	Pellicular ion exchangers	Horvath, Kirkland	
1975	Ion exchange chromatography with conductivity detection using a "stripper"	Small, Stevens, Baumann	**HPLC**
1979	Conductivity detection without a "stripper"	Gjerde, Fritz, Schmuckler	
1976-80	Ion pair chromatography	Waters, Bidlingmeier, Horvath *et.al*	

Today, there are many important fields of application for ion chromatography such as the routine investigation of aqueous system, analysis of ions in chemical products, foods, cosmetics, ultra-trace analysis.

Fig.1-2 The schematic of an ion chromatographic system



The above schematic represents a non-suppressed ion chromatography system. The sample is introduced onto the system via a sample loop on the injector. In the inject position the sample is pumped with the eluent onto the column. The sample ions are attracted to the charged stationary phase of the column. The charged eluent elutes the retained ions which then go through the detector and are depicted as peaks on a chromatogram.

1.5 Modification of stationary phase

Generally, chemical modification of the stationary phase is carried out to change phase selectivity. It is possible to take advantage of certain characteristics of the stationary phase and apply it to a different mode chromatography.

One of the modifications of the stationary phase is using surfactant. Surfactants have hydrophobic and hydrophilic centers both of which affect their physical and chemical properties. There are two types of surfactants: anionic surfactant and cationic surfactant. Anionic surfactants are classified as alkanesulfonates, alkyl sulfates and alkylbenzene sulfonates. The cationic surfactants are quarternary ammonium compounds [22].

This modification technique has been utilized for the analysis of inorganic ions in both particulate and monolithic reversed-phase columns [23-25]. Surfactant layer monolithic reversed

phase column allows higher flow rates and higher efficiency than the stationary phase to apply to IC. Unfortunately, this modification is not permanent, the coating can be lost from the column and thus affect of the retention time.

Another modification of stationary phase is attained by using chemical modifications. Rahmah *et al.* [26] modified the polymer monolithic column (poly GMA/PEGDMA) in order to separate the inorganic anions. The modification was performed by attaching diethylamine to make anion exchanger. Siswoyo and co-workers modified the silica monolithic columns for separation of gold nano particles [27].

1.6 Detection in capillary liquid chromatography

One of the essential in order to get a good separation is detector. Therefore, many of detection systems have been developed for LC.

Table 1-5 Typically used detectors for HPLC [28]

Detector	Limit of detection (g)
Conductivity detector	10^{-6}
Refractive index detector	10^{-7}
UV detector	10^{-10}
Fluorescence detector	10^{-12}
Amperometric detector	10^{-12}
Coulometric detector	10^{-12}

Table 1-5 shows the type of detector based on their sensitivity and also commonly used in LC. Detector can be classified into two general categories: selective detector and universal detector. Selective detector has a good sensitivity, low background signal and it is good for limited species. In contrast, the universal detector has a poor sensitivity, high background signal and it is good for unlimited species. UV, fluorescence, polarographic, amperometric and coulometric are included in the selective detectors. Refractive index detector is involved in universal detector [28].

1.7 Objective of the present research

HPLC has been widely used to determine many species of interest in various fields such as biology, industry, environment, pharmacology, *etc*. [29]. Various eluent-induced stationary phases also have been developed in HPLC. In the literature, several analytical methods have been introduced for separation and determination of ion especially inorganic cations. Some of the eluent-induced methods have been introduced for ionic separation such as ion-pair chromatography [30], ion-interaction chromatography [31], soap chromatography [32], *etc*. Crown ether as eluent additive was used to separate inorganic anions [33].

Chapter 2 describes the use of perfluoroalkanesulfonic acids (PFOS) as an eluent additive for separation of inorganic cations on hydrophobic stationary phase (C30) by using a UV detector. The reason using this eluent was because it was commercially available in acid form. In this time, copper sulfate was used as the co-additive in the indirect detection of the cations.

Chapter 3 describes a simple and easy preparation for separation of inorganic cations using C30 stationary phase by using a contactless conductivity detector. Methanesulfonic acid and a

small concentration of SDS were used as eluents in this system. SDS has a hydrophobic tail, which could be associated with the C30 alkyl-chain bonded to the silica stationary phase and the sulfate head group oriented away from the surface that can be a cation-exchange site.

Chapter 4 describes polymer monolithic columns which were used for separation of inorganic cations by using contactless conductivity detector. Glycidyl methacrylate (GMA) and PEGDMA were used as monomer and crosslinker, respectively. Methanol and decanol were used as binary porogenic solvents. The monolith columns were prepared by two step procedures: polymerization and sulfonation of GMA.

Chapter 5 describes one-pot synthesis of polymer monolithic columns which were used for separation of inorganic cations by using contactless conductivity detector. 2-Acrylamide-2-methylpropanesulfonic acid (AMPSA) and EDMA were used as monomer and crosslinker, respectively. 1-Propanol, 1,4-butanediol and methanol were used as porogenic solvents. AMPSA has a sulfonic functional group that can be a cation-exchange site.

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Chapter 2

Separation of inorganic cations on a hydrophobic stationary phase using perfluoroalkanesulfonic acid as the eluent additive in ion chromatography

2.1 Introduction

Various stationary phases have been developed in ion chromatography since it was initiated by Small *et al.* [1]. In most cases, ionic species are separated *via* electrostatic attraction or repulsion between analytes and the stationary phase by using ion-exchange columns. Reversed phase liquid chromatography can also separate ionic species where a hydrophobic reagent with a charge is contained in the eluent to separate ionic analyte components with the opposite charge. These eluent-induced methods for the separation of ionic species have been proposed using various names [2], involving ion-pair chromatography [3], ion-interaction chromatography [4], dynamic solvent-generated ion-exchange chromatography [5], soap chromatography[6], etc. The eluent-induced separation provides some advantages over the separation methods using common ion exchangers, such as achievement of better column efficiency and selectivity by selection of the stationary phase, eluent and/or additive as well as variable ion-exchange capacity by selection of the eluent composition. Our recent work used 18-crown-6 ether (18C6) as the eluent additive in the reversed-phase mode to separate inorganic anions, where the eluent cation was

trapped on 18C6, which was adsorbed on the stationary phase, worked as the anion-exchange site [7].

The present paper examines perfluoroalkanesulfonic acid as the eluent additive in the reversed phase mode to separate inorganic cations. Perfluoroalkanesulfonic acids were selected because some perfluoroalkanesulfonic acid reagents are commercially available in acid form.

2.2 Material and Methods

2.2.1 Reagents and materials

Perfluorooctanesulfonic acid (PFOS) and perfluorobutanesulfonic acid (PFBS) were obtained from Wako Pure Chemical Industries (Osaka, Japan). Purified water was produced in the laboratory by using a GS-590 water distillation system (Advantec, Tokyo, Japan). Methanol was of HPLC-grade and obtained from Kanto Chemical (Tokyo, Japan). All solutions used in this work were prepared using purified water. Other reagents employed were of guaranteed reagent grade and were obtained from Wako Pure Chemical Industries. The stationary phase examined in this work was Develosil C30-UG-5 (C30, 5 μm particle diameter; Nomura Chemical, Seto, Japan). The packing material was packed into fused-silica tubing with 0.53 mm i.d., as previously reported [8].

2.2.2 Apparatus

The chromatographic measurements were carried out by 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 (0.5mL; Ito, Fuji, Japan) as a pump, a model M435 microinjection valve with an injection volume of 0.15 μL (Upchurch Scientific, Oak Harbor, WA, USA) as an injector, a 0.53 mm i.d.×100 mm microcolumn, and a UV- 2070 UV detector

(JASCO, Tokyo, Japan). The UV detector was operated at 210 nm. A capillary flow cell (75 μm; JASCO) was attached to the UV detector. The data were acquired by a Chromatopac C-R4A data processor (Shimadzu, Kyoto, Japan). The inlet pressure was monitored by an L.TEX-8150 Pressure Sensor (L.TEX). The separation column was dipped in a water bath and left ambient. The measurements were carried out at room temperature (21-22°C).

2.3 Results and Discussion

2.3.1 Effect of methanol concentration

Inorganic cations were separated on a capillary column packed with C30 by using aqueous methanol solution containing 1 mM PFOS and 1 mM copper sulfate, where copper sulfate was used for indirect photometric detection of analyte cations. The methanol concentration in the eluent affected the adsorption of PFOS on the C30 stationary phase. It is expected that the lower the methanol concentration, the more the amount of PFOS dynamically adsorbed on the stationary phase will be, leading to the increase in the retention of analyte cations. Figure 2-1 illustrates the retention time of the analyte cations as a function of the methanol concentration in the eluent. It can be seen that the retention time of the monovalent cations decreased with increasing methanol concentration in the eluent. A system peak appeared between sodium ion and ammonium ion. It should be noted that both copper and hydronium ions are driving ions in the present system.

Figure 2-2 demonstrates the separation of monovalent cations on the C30 stationary phase using 25 – 33% methanol solutions. It is found that good resolution is achieved for the 25 and 27% methanol eluents. The system peak appeared between sodium and ammonium ions, and it overlapped with ammonium ion when the methanol concentrations were 30 and 33%. The

system peak denoted as “S” or “Sys” may be related with proton, which will be discussed thereinafter. It can be seen that the signal intensities of the analytes eluting before the system peak are smaller than those of the analytes eluting after the system peak. It should be noted that the resolution between sodium ion and ammonium ion is improved, compared with that observed in common ion chromatography [9].

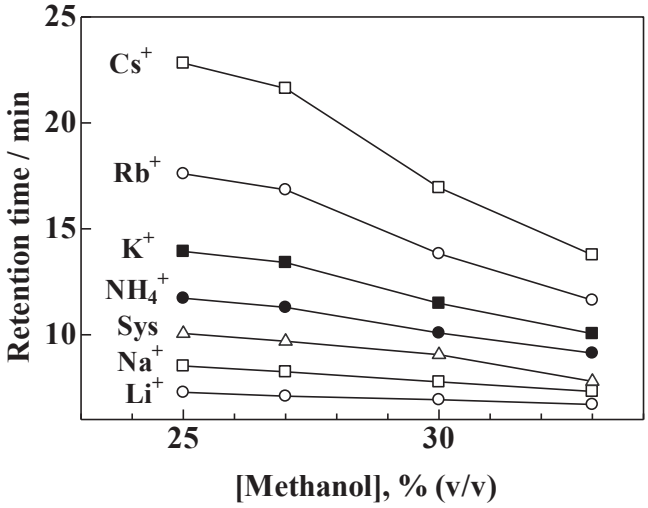

Fig. 2-1 Retention of inorganic monovalent cations as a function of methanol concentration in the eluent. Column, Develosil C30-UG-5 (C30), 100×0.53 mm i.d.; eluent, aqueous methanol solution including 1 mM PFOS and 1 mM copper sulfate; flow-rate, 8.0 μL min^{-1}; Sys, system peak; concentration of analytes, 1.0 mM each; injection volume, 0.15 μL; wavelength of UV detection, 210 nm.

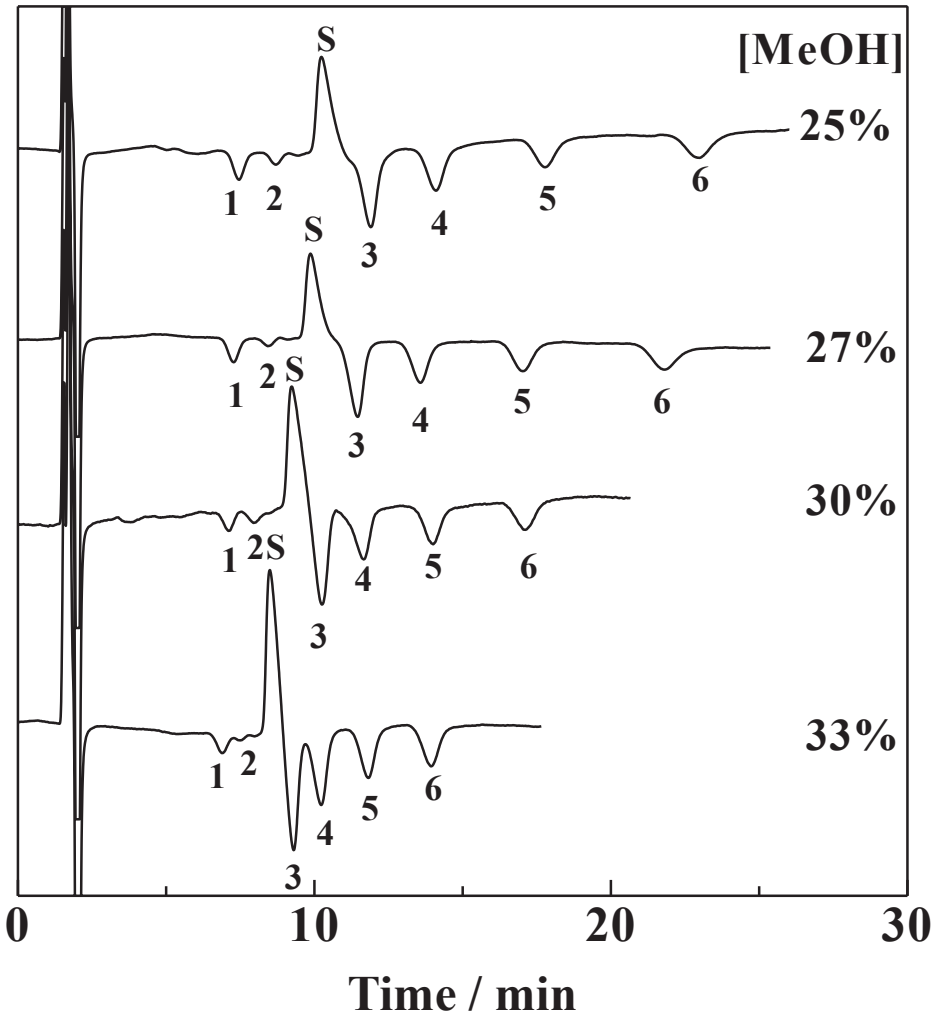

Fig. 2- 2 Separation of monovalent cations on C30 using different concentrations of methanol as the eluent. Analytes, 1=lithium, 2=sodium, 3=ammonium, 4=potassium, 5=rubidium, 6=cesium ions, S=system peak; other operating conditions as in Fig. 2-1.

Table 2-1 shows the peak areas for various methanol concentrations, where the copper sulfate and PFOS concentrations are kept at 1 mM each, respectively. Although it can be seen that the methanol concentration affects the peak area, it was not possible to find some regularity in the variation of the peak area.

Table 2-1 Effect of methanol concentration on peak area of analyte cations. Operating conditions are as in Figure 2-1. Peak areas are given in arbitrary units.

Analyte	Methanol concentration			
	25%	27%	30%	33%
Li^+	-55	-46	-36	-33
Na^+	-18	-11	-21	-5
NH_4^+	-199	-213	N.A	N.A
K^+	-135	-115	-133	-160
Rb^+	-119	-99	-122	-138
Cs^+	-132	-111	-106	-131

N.A. = non applicable

2.3.2 Effect of copper sulfate concentration

The retention time of inorganic monovalent cations as a function of the copper sulfate concentration in the eluent is shown in Figure 2-3. The eluent employed in Figure 2-3 is 27% methanol (v/v) aqueous solution containing 1 mM PFOS and copper sulfate with different concentrations as indicated. It can be seen that the lower the copper sulfate concentration, the longer the retention time of the analyte cations is. The results in Figure 2-3 are reasonable since copper ion is a driving ion.

Figure 2-4 demonstrates the separation of monovalent cations on the C30 stationary phase using 27% methanol solutions containing 1 mM PFOS and copper sulfate with different concentrations as indicated. It is found that the system peak overlapped with ammonium ion or sodium ion, depending on the copper sulfate concentration except for 1 mM. The best resolution is obtained for the 1 mM copper sulfate concentration among the conditions examined in this work.

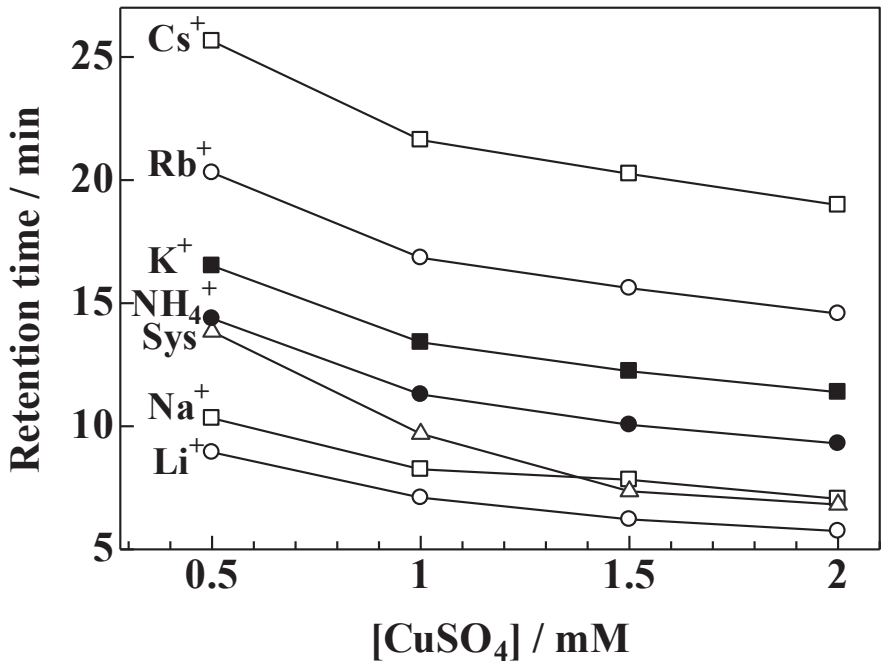

Fig. 2-3 Retention of inorganic monovalent cations as a function of copper sulfate concentration in the eluent. Eluent, methanol-water mixture (27:73, v/v) including 1 mM PFOS and copper sulfate as indicated; other operating conditions as in Fig. 2-1.

Table 2-2 shows the effect of the copper sulfate concentration in the eluent on the peak area of the analyte cations. It was observed that lower concentrations of copper sulfate in the eluent had a tendency to provide larger peak areas.

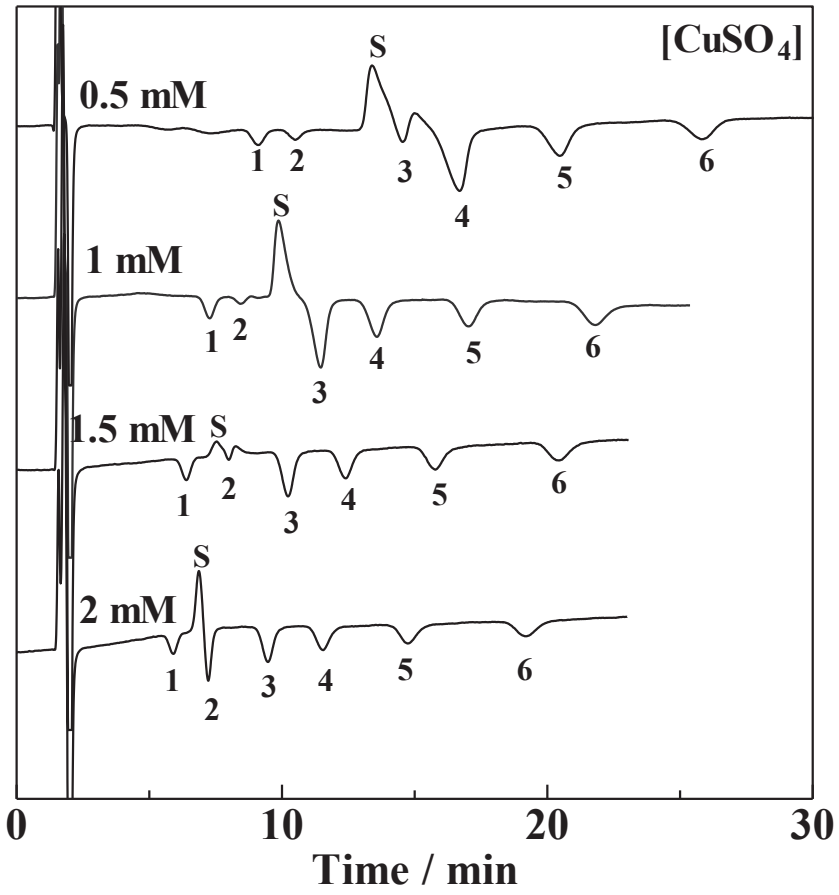

Fig. 2-4. Separation of monovalent cations on C30 using different concentrations of copper sulfate as the eluent. Concentration of copper sulfate, as indicated; analytes, as in Figure 2-2; other operating conditions as in Figure 2-3.

Table 2-2 Effect of copper sulfate concentration on peak area of analyte cations. Operating conditions are as in Figure 2-3. Peak areas are given in arbitrary units.

Analyte	Copper sulfate concentration			
	0.5 mM	1.0 mM	1.5 mM	2.0 mM
Li^+	-42	-46	-49	-37
Na^+	-28	-11	N.A.	N.A.
NH_4^+	N.A.	-213	-131	-98
K^+	-329	-115	-96	-80
Rb^+	-164	-99	-105	-79
Cs^+	-103	-111	-98	-74

N.A. = non applicable

2.3.3 Effect of PFOS concentration

The retention time of inorganic monovalent cations as a function of the PFOS concentration in the eluent is shown in Figure 2-5. The eluent employed in Figure 2-5 is 27% methanol (v/v) aqueous solution containing 1 mM copper sulfate and PFOS with different concentrations as indicated.

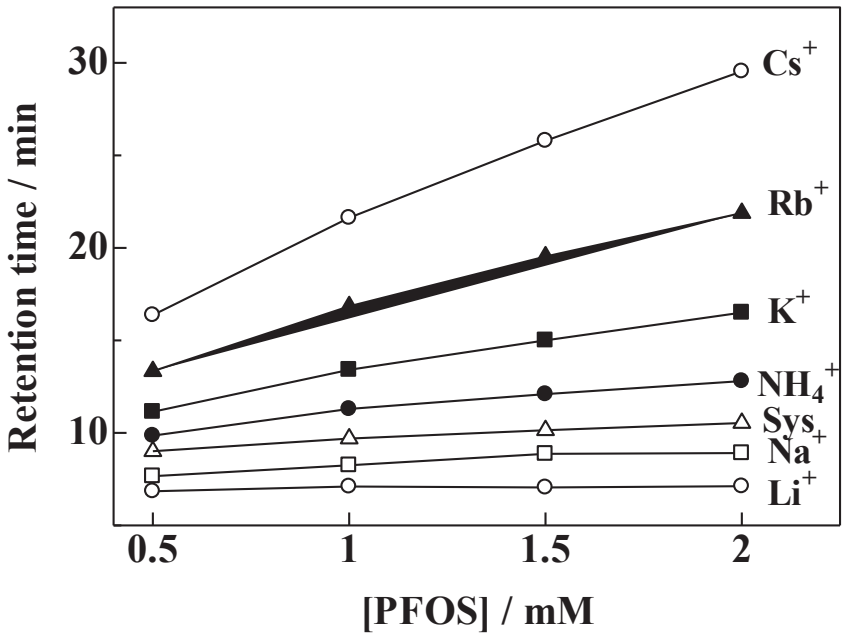

Fig. 2-5 Retention of inorganic monovalent cations as a function of PFOS concentration in the eluent. Eluent, methanol-water mixture (27:73 v/v) including 1 mM copper sulfate and PFOS as indicated; other operating conditions as in Figure 2-1.

It can be seen that the retention time of the analyte cations increased with increasing PFOS concentration. The PFOS concentration could have two competing influences on the retention of the analyte cations. The larger the PFOS concentration, the larger the amount of PFOS adsorbed on the hydrophobic stationary phase, leading to the increase in the retention of the analyte cations. On the other hand, the increase in the PFOS concentration in the eluent leads to the increase in the elution strength, leading to the decrease in the retention. The results in Figure 2-5 indicate that the former contribution surpasses the latter contribution.

Figure 2-6 demonstrates the separation of monovalent cations on the C30 stationary phase using 27% methanol solutions containing 1 mM copper sulfate and PFOS with different

concentrations as indicated. It is found that the retention of the analyte cations as well as the system peak increased with increasing PFOS concentration in the eluent. At the PFOS concentrations higher than 1 mM, baseline separations were achieved for all of the monovalent cations. In addition, a small positive peak was observed for sodium ion when the PFOS concentration was 2 mM. The reason for this phenomenon is not elucidated. Table 2-3 shows the effect of the PFOS concentration in the eluent on the peak area of the analyte cations. It was observed that lower PFOS concentrations in the eluent had a tendency to provide larger peak areas. This can be explained by the fact that copper ion and proton are driving eluent cations and copper ion could contribute more strongly with decreasing PFOS concentration, leading to better efficiency of indirect photometric detection.

In addition, divalent cations such as magnesium and calcium could not be eluted from the column under the operating conditions examined above.

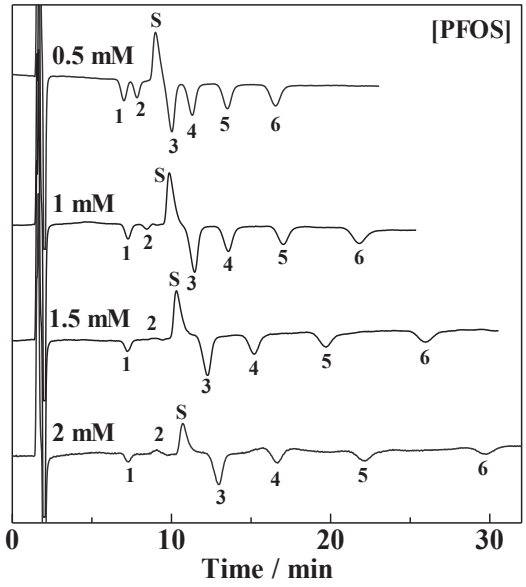

Fig. 2-6 Separation of monovalent cations on C30 using different concentrations of PFOS as the eluent. Concentration of PFOS, as indicated; analytes, as in Fig. 2-2; other operating conditions as in Figure 2-5.

Table 2-3 Effect of PFOS concentration on peak area of analyte cations Operating conditions are as in Figure 2-5. Peak areas are given in arbitrary unit.

Analyte	PFOS concentration			
	0.5 mM	1.0 mM	1.5 mM	2.0 mM
Li^+	-67	-46	-40	-29
Na^+	-46	-11	-4	16
NH_4^+	N.A.	-213	-128	-166
K^+	-137	-115	-127	-97
Rb^+	-127	-99	-96	-90
Cs^+	-129	-111	-101	-68

N.A. = non applicable

2.3.4 Effect of acidity of the sample

It was expected that the system peak was related with the acidity of the sample solution. Figure 2-7 illustrates the signal intensity (peak height) of the system peak when water or HCl solutions with different concentrations were injected into the system, where methanol-water mixture (27:73 v/v) including 1 mM copper sulfate and 1 mM PFOS (pH 3.0) was used as the eluent. The system peak was positive when water was injected, whereas the system peaks were negative 0.5-2 mM HCl solutions were injected. It can be seen from the figure that the peak height of the negative system peak increased with increasing HCl concentration injected. This result would imply that the present system could evaluate the acidity of the sample solution.

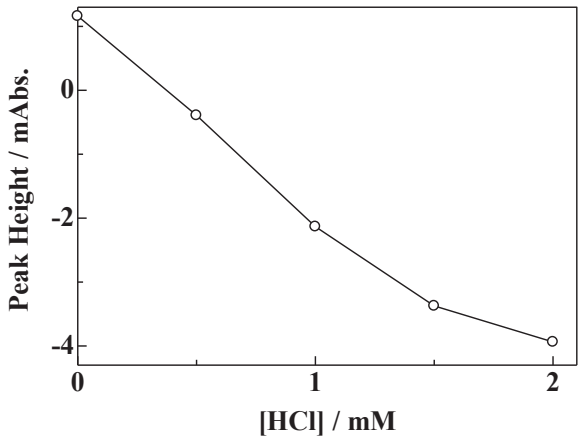

Fig. 2-7 Effect of acidity on the signal intensity of the system peak. Eluent, methanol-water mixture (27:73 v/v) including 1 mM copper sulfate and 1 mM PFOS (pH 3.0); sample, water or HCl with different concentrations; other operating conditions as in Figure 2-1.

2.3.5 PFBS as eluent additive concentration

PFBS was also evaluated as the eluent additive for the separation of cations. The retention of cations could be controlled by the concentration of methanol, copper sulfate and PFBS. Since

PFBS is less hydrophobic, the methanol concentration should be decreased, compared with the case for PFOS. Figure 2-8 demonstrates the separation of sodium and magnesium ions using methanol water mixture (10:90, v/v) including 1 mM copper sulfate and 1 mM PFBS as the eluent. It can be seen that these cations with different charges are eluted from the column under the condition in Figure 2-8. It should be noted that other monovalent cation eluted close to sodium ion, whereas calcium ion eluted at the same retention time of magnesium. It was found that the selectivity for monovalent cations as well as for divalent cations was poor. It is speculated that the sulfo group of PFOS is less hydrated, compared with that of PFBS, leading to the decrease in the distance between the sulfo group and analyte cations when they are interacted. The decrease in the distance between the sulfo group and analyte cations could in turn lead to the increase in the selectivity of analyte cations.

In addition, various peaks due to the eluent components also appear in the chromatogram in Figure 2-8. In addition, perfluoroalkanesulfonic acids other than PFOS and PFBS were not commercially available, unfortunately.

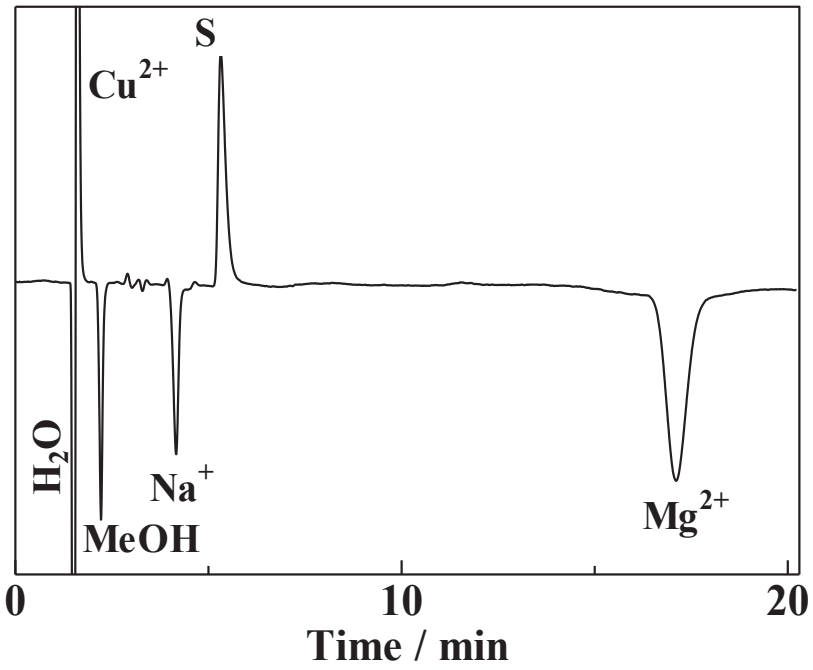

Fig. 2-8 Separation of cations on C30 using PFBS as the eluent additive. Eluent, methanol-water mixture (10:90 v/v) including 1 mM copper sulfate and 1 mM PFBS; analytes, 1 mM each of sodium and magnesium ion; other operating conditions as in Figure 2-1.

2.4 Conclusions

Li^+, Na^+, NH_4^+, K^+, Rb^+ and Cs^+ were separated with good selectivity on the C30 stationary phase by using PFOS as the eluent additive, where copper sulfate as the co-additive in the eluent allowed the indirect detection of the cations. PFBS as the eluent additive provided poor selectivity although monovalent and divalent cations were eluted under the same eluent conditions. It is expected that the use of conductivity detection will simplify the eluent conditions.

2.5 References

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Chapter 3

Eluent-induced separation of inorganic cations in capillary liquid chromatography with contactless conductivity detector

3.1 Introduction

Ion chromatography (IC), introduced by Small *et al.* in 1975 [1], gave the first feasible analytical method for the simultaneous determination of trace inorganic anions, and it was then developed to also give convenient means for the separation of cations [2-4]. In 1983, Rokushika *et al.* firstly reported the use of capillary tubes in their IC system [5] and after that the miniaturization era has begun and capillary IC has proved itself to be a useful technique for the separation of trace inorganic and organic ions in clinical [6] and environmental samples [7-9]. Among its many advantages, capillary IC could improve mass sensitivity and reduce consumptions of stationary phase, mobile phase and sample volumes of high matrix complexity [10,11].

Since Sanders and Wise [12] firstly published C30 column in 1987, it has been increasingly applied for determination of various caretonoids [13], bioactive compounds [14], analysis of food [15] by liquid chromatography (LC) attributed to its better selectivity than ODS (C18) columns. Most of the ionic species can be separated *via* electrostatic attraction or repulsion between stationary-phase and analytes by using ion-exchange column. C30 can also be modified with polyoxyethylene oleyl ether [16] and hexadimethrine bromide [17] for determination of

inorganic anions. Qun Xu *et al.* employed ion chromatographic determination of cations by C30 dynamically coated with dodecylsulfate and 18-crown-6-ether using ethylenediamine solutions containing small concentration of lithium dodecylsulfate (Li-DS) and 18-crown-6-ether as the eluent by conductivity detector [18].

Conductivity detection has been a simple and universal detection technique in IC because it allows the detection of many kinds of ionized species with good sensitivity [19-21]. Over the past several years, a new version of conductivity detection known as contactless conductivity detection (CCD) [22] has been commercially available and attracted a great deal of interest especially for cations analysis. Sprung *et al.* developed online-system analysis for metal cations by combining flow injection with capillary electrophoresis by using CCD [23]. Another researchers also used CCD for simultaneous separation of inorganic anions and cations [24]. CCD combination with capillary liquid chromatography has extended a sensitive detection for many various samples [25-27].

Some of the eluent-induced methods have been introduced for ionic separation such as ion-pair chromatography [28], ion-interaction chromatography [29], crown ether as eluent additive [30], *etc*. In this study, we report the use of the suitable chemical additives that contained a strong cation exchanger for examples sulfonic acid and manipulate the eluent composition by using contactless conductivity detector for the separation of inorganic cations *via* single column capillary IC. The retention behavior of inorganic cations on modified C30 column was investigated under various eluent compositions.

Our previous work examined perfluoroalkanesulfonic acid as the eluent additive in the reversed-phase mode to separate inorganic cations by using indirect UV detection where the eluent additive was adsorbed on the stationary phase, worked as cation-exchange site [31].

The dynamic modification of octadecylsilica column with dodecylsufate has been used by Haddad et al. [33] for separation of hydronium with an eluent comprising 0.3mM LDS, 50 mM KCl and 0.1 mM H_2SO_4 and the aggregates structure formed by the molecules of the dodecylsulfate at the surface of the stationary phase was influenced by the concentration of SDS, KCl, and the acidity of the eluent. Ito et al. reported the use of C18 modified with SDS by using 0.8 mM Ce(III), 0.1 mM SDS and 1mM HNO_3 as eluent for separation and determination of alkali- and alkaline earth and ammonium ions [4].

In our research, we tried to develop a stationary phase for simple and direct separation of inorganic cations using a laboratory-made C30 packed stationary phase and methanesulfonic acid solution as eluent. We believed that the eluent can adsorbed into the stationary phase. And also tried to do modification on the C30 column, the column coated with surfactant (SDS) obtained by pumping the variation of concentration from 1, 3, 5 and 7% or 35 -240 mM. SDS has a hydrophobic tail, which could be associated with the C30 alkyl-chain bonded to the silica stationary phase and the sulfate head group oriented away from the surface that it can be cation-exchange site. The concentrations of SDS were far above from the critical micelle concentration of SDS at 25 ^{o}C (8.3 mM), in which surfactant monomers aggregate to form micelle. It is presumed that C30 stationary phase coated with the micelle aggregates of SDS.

Since C30 is more hydrophobic than C18, it is expected that SDS adsorbed on the C30 stationary phase will be more stable than that on the C18 stationary phase. Another advantages of

C30 stationary phase over C18 column the retention time of analytes is stable even when the aqueous solution is used because little aqueous eluent is excluded from the mesopores of C30 packing material during the operation.

3.2 Experimental

3.2.1 Apparatus

All experiments were assembled with 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 injector with an injection volume of 0.2 μL (Rheodyne, Cotati, CA, USA) as an injector, a 0.32 mm i.d. × 100 mm microcolumn, and a Tracedec contactless conductivity detector (Istech, Strasshof, Austria). The flow-rate of the pump was kept at 4 μL min^{-1}. The data were acquired by a Chromatopac C-R7Ae plus data processor (Shimadzu, Kyoto, Japan). The separation column was immersed in a water bath. The measurements were carried out at room temperature (25 ˚C).

3.2.2 Reagents and materials

Methanesulfonic acid (MSA) was employed from Nacalai Tesuque (Kyoto, Japan) and Sodium Dodecylsulfate (SDS) was obtained from Wako Pure Chemical Industries (Osaka, Japan). Purified water was produced in the laboratory by using a Millipore Simplicity UV system (Darmstadt, Germany). All solutions used in this work were prepared using the purified water. Other reagents employed were of guaranteed reagent grade and were obtained from Wako Pure Chemical Industries. SDS was used for coating the columns and as the components of eluents.

3.2.3 Column preparation

3.2.3.1 Separation column

Develosil C30-UG-5 (C30; 5 μm particle diameter; Nomura Chemical, Seto, Japan) packing materials were taken from conventional-size packed columns commercially available, and were packed into a fused-silica capillary with 0.32 mm i.d. by using a slurry packing method, as previously reported [32] and then conditioned with purified water. An aqueous solution containing SDS was then passed into the fused-silica capillary at a flow-rate of 4 μL/min for *ca.* 2h. The concentration of SDS dissolved in water as the modification solution was examined. The column was operated at room temperature (*ca.* 25^{o}C).

3.2.3.2 H^+ exchange column

As for the case of SDS as the eluent additive, an H^+-cation exchange column was placed before the sample injector to convert the Na^+ from the eluent into H^+. Oasis MCX sorbent (Sulfonic Acid, 1 meq/g; 29.3 μm particle diameter ; Waters, Milford, USA) packing materials were taken from cartridge commercially available and also packed into a fused-silica capillary 0.53 mm i.d. using a slurry packing method and the conditioned with purified water. A schematic diagram of instruments is shown in Fig. 3-1.

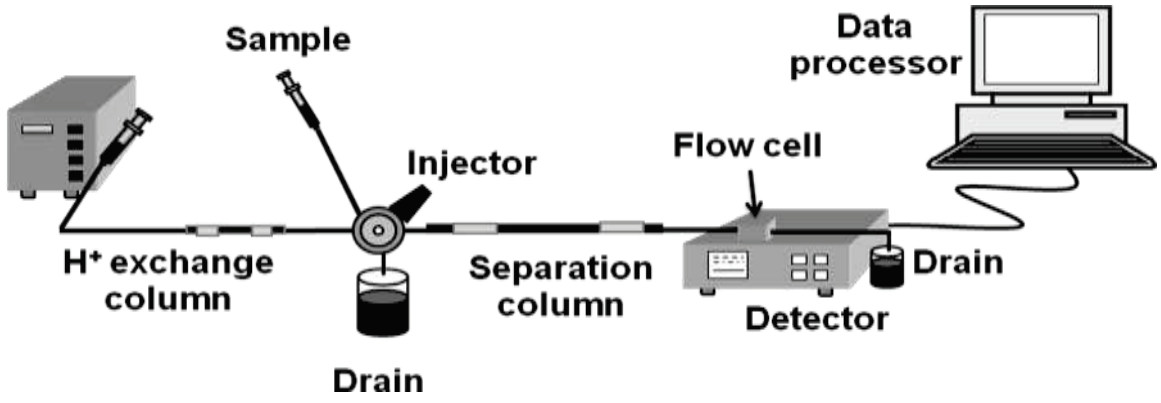

Fig. 3-1 Schematic diagram of instrument

3.3 Results and Discussion

3.3.1 Effect of modification conditions

C30 stationary-phase modified with dodecyl sulfate, is well-established and convenient way to create stationary phase with sulfate groups for separating inorganic cations. Mixtures of MSA and SDS solution were chosen to separate monovalent cations as the eluent. When a sample was injected into the column, the sample was displaced with the eluent that contained MSA and docecylsulfuric acid, and the retained cations were then eluted by further MSA and docecylsulfuric acid supplied by the eluents.

Fig. 3-2 demonstrates separation of inorganic cations on a capillary column packed with C30 modified with SDS by using the mixture of 40 mM MSA and 0.25 mM SDS as the eluent. The effect of the SDS concentration coated onto the C30 column was examined. The SDS concentrations used were 1, 3, 5, and 7% (equivalent to 35-240 mM), respectively. The concentration of SDS used were above from the critical micelle concentration of SDS in 25 ^{o}C

(*i.e.*, 8.3 mM), in which surfactant monomers aggregate to form micelles, and it is presumed that C30 stationary phase was coated with the micelle aggregates of SDS. Without coating of SDS, it is not possible to retain and/or detect cations using MSA or SDS as the eluent. In the other words the coating of SDS micelle aggregate was crucial in maintaining sufficient cation-exchange site for separation. When the C30 column was coated with the 5 % SDS, good separation of monovalent cations was achieved. The resolution (*Rs*) between ammonium and potassium ions in the 5 % of SDS concentration was 1.72. Since the C30 column coated with 5 % gave the best resolution, C 30 columns were coated with 5 % of SDS in the following experiments.

From the Fig. 3-2, a small peak seemed to be eluted in front of potassium ion peak approx. 13 min as can be seen from the chromatogram obtained with 3 % SDS. This small peak could be hydronium (H^+) peak as H^+ could be detected under acidic condition [4, 32]. As for the systems described previously [18,32], the use of eluent pH between 4.0-6.8 could detect H^+ ; however, the combination study of 40mM MSA and 0.25 mM SDS used in this study has pH value of *ca.* 3, in addition, H^+ peak could not be detected. Nevertheless, the identity of this small peak is not yet to be determined.

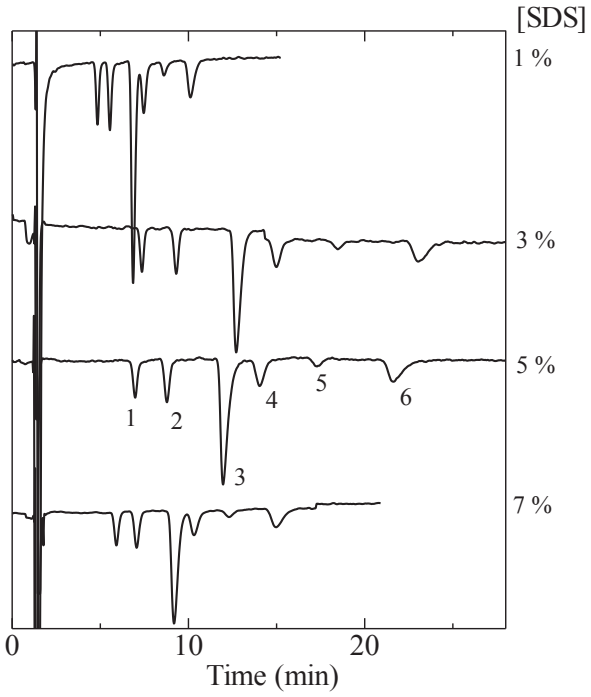

Fig. 3-2 Separation of an authentic mixture of six cations on the modified Develosil C30-UG-5 column modified with various concentration of SDS. Column, 100 × 0.32 mm i.d.; eluent, 40 mM methanesulfonic acid and 0.25mM sodium dodecylsulfate; flow-rate, 4 μL min^{-1}. Analytes (concentration in mM), 1=Li^+ (1.0), 2=Na^+ (1.0), 3=NH_4^+ (1.5), 4=K^+ (1.0), 5=Rb^+ (1.0), 6=Cs^+ (1.0); injection of volume, 0.2 μL; detection, contactless conductivity.

3.3.2 Effect of methanesulfonic acid concentration in the mobile phase

The effect of MSA concentration in the mobile phase was investigated for six inorganic monovalent cations. Experiments showed that with increasing the MSA concentration in the eluent, the retention time of Li^+, Na^+, NH_4^+, K^+, Rb^+ and Cs^+ decreased and the background conductivity increased as shown in Fig. 3-3. When the concentration of MSA exceeded 40 mM, each monovalent cation was not separated adequately from the other monovalent cations, and

also the detection sensitivity decreased because of the high background conductance. This means that the hydronium ion in MSA eluent competes with analyte ion for the cation-exchange site. As expected the peak height decreases with increasing retention time. Considering the retention time and the sensitivity, 40 mM MSA and 0.25 mM SDS were selected as the eluent for the following experiments described below.

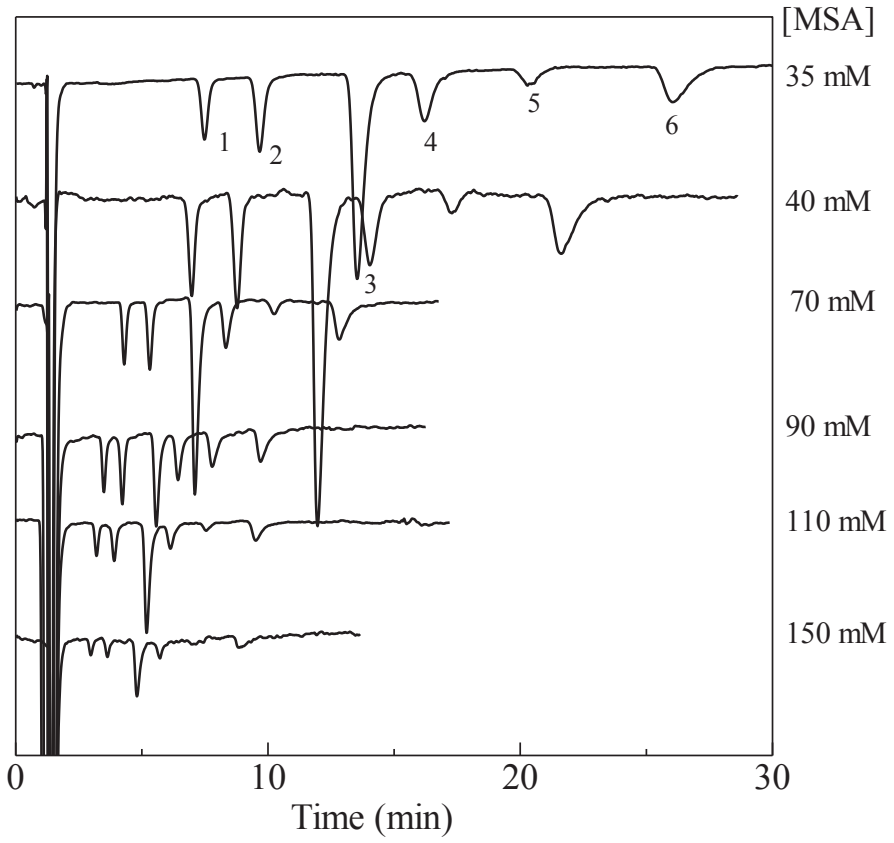

Fig. 3-3 Separation of monovalent cations on C30 coated with SDS ; 100 × 0.32 mm i.d.; eluent, different concentrations of MSA; concentration of SDS, 0.25 mM; flow-rate, 4 μL min^{-1}; analytes (concentration in mM), 1=Li^+ (1.0), 2=Na^+ (1.0), 3=NH_4^+ (1.5), 4=K^+ (1.0), 5=Rb^+ (1.0), 6=Cs^+ (1.0) ; injection volume, 0.2 μL; detection, contactless conductivity detector.

Almost linear relationships between log *k* and log [MSA] were obtained for monovalent cations under investigation as shown in Fig. 3-4. The slopes for the monovalent cations were -0.98, -0.93, -0.88, -0.85, -0.82, -0.78 for Li^+, Na^+, NH_4^+, K^+, Rb^+, Cs^+, respectively; showing that later eluted cations would be slightly influenced by the hydrophobic interaction with the stationary phase rather than pure ion-exchange mechanism.

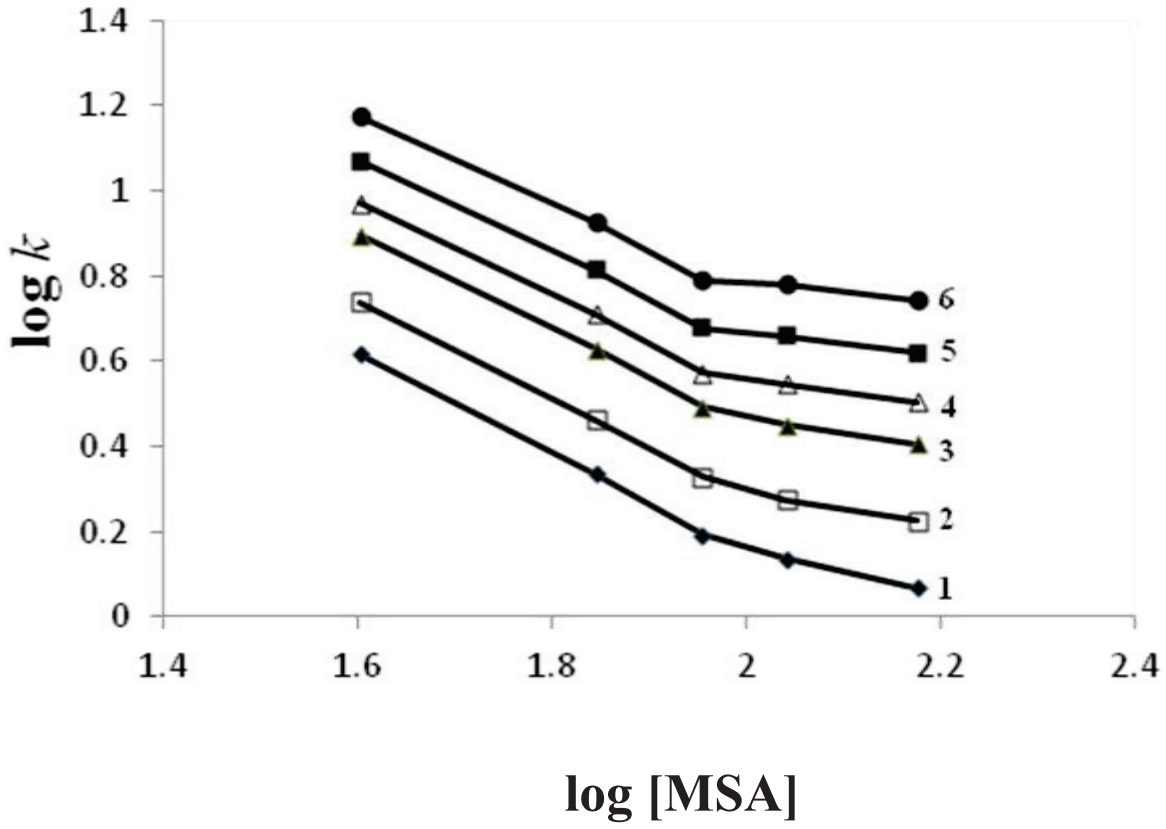

Fig. 3-4 The logarithm of the retention factor versus the logarithm of the MSA concentration for monovalent cations. Eluent, 35-150mM MSA; column, C30 coated with 5% SDS (100 x 0.32 mm i.d.); eluent flow rate, 4 μL min^{-1}; column temperature, room temperature; injection volume, 0.2μL; plot lines, 1=Li^+; 2=Na^+; 3=NH_4^+; 4=K^+; 5=Rb^+; 6=Cs^+.

3.3.3 Effect of SDS concentration in the mobile phase

Separation of the six monovalent cations on the C30 column modified with 5% SDS using 40 mM MSA and SDS with different concentration as shown in Fig.3-5.

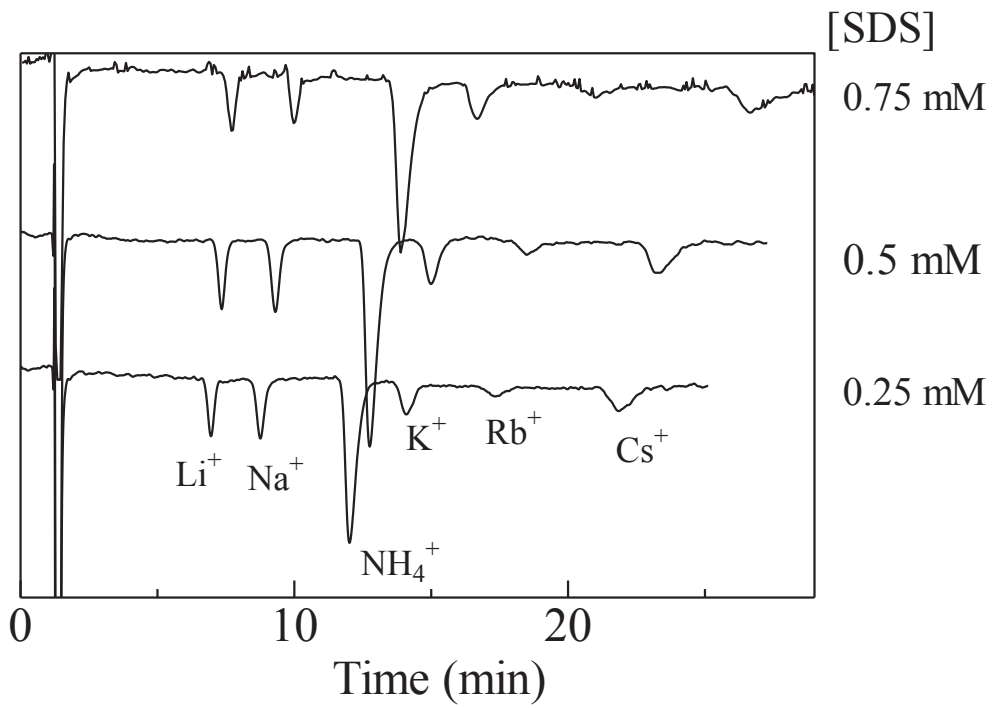

Fig. 3-5 Separation of monovalent cations on C30 coated with 5% SDS. 100 × 0.32 mm i.d.; eluent, different concentrations of SDS; concentration of MSA, 40 mM; concentration of SDS, as indicated; flow-rate, 4 μLmin^{-1}; analytes (concentration in mM), 1=Li^+ (1.0), 2=Na^+ (1.0), 3=NH_4^+ (1.5), 4=K^+ (1.0), 5=Rb^+ (1.0), 6=Cs^+ (1.0); injection volume, 0.2 μL; detection, contactless conductivity detector.

It is found that increasing the concentration of SDS from 0.25 to 0.75 mM slightly increases the retention time, as shown in Fig. 3-6. From the chromatogram, it can be seen that the peak

height is almost the same, independent of the SDS concentration. Therefore, 0.25 mM SDS was selected as the optimum concentration in this study. Table 1 shows the relative standard deviations (RSDs) of retention time without SDS in the eluent, it is found the RSD is larger than 4.5%. Because of that, a little concentration of SDS was incorporated in the eluent to replace any SDS lost from the column and it was only supplement to the stationary phase.

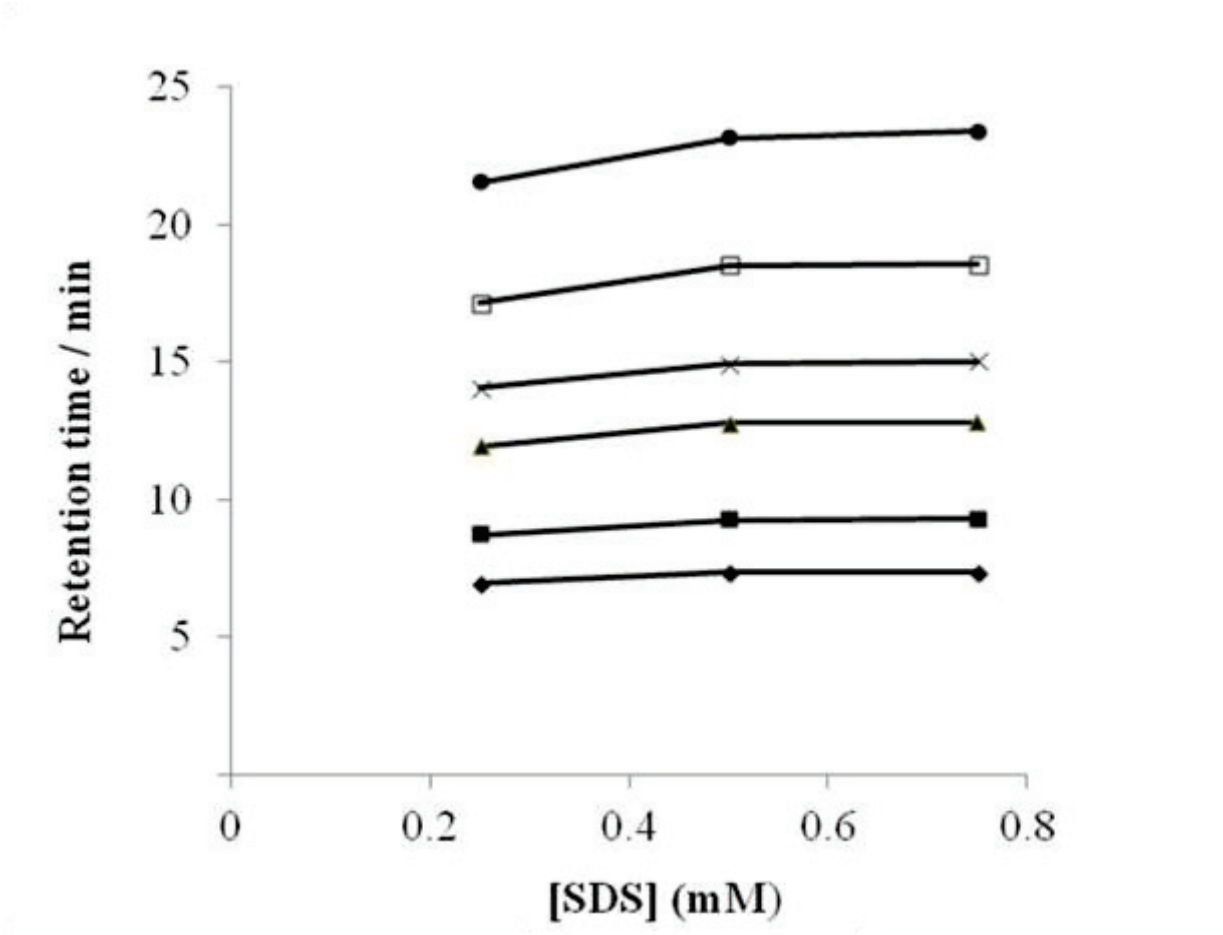

Fig. 3-6 Retention of inorganic monovalent cations as a function of SDS concentration in the eluent. Column, Develosil C30-UG-5 (C30), 100 × 0.32 mm i.d.; eluent, SDS solution including 40 mM MSA; flow-rate, 4.0 μL min^{-1}; concentration of analytes, 1.0 mM each; injection volume, 0.2 μL; detection, contactless conductivity detector.

Compared to the other types of liquid chromatographic separation, ion-exchange separations in our work gave a better selectivity than the conventional system, even though the limit of detection (LOD) was a little bit poor. Table 3-2 summarized data for column efficiency, RSDs of the retention time and peak height, LOD of inorganic cations obtained under optimum operating condition as in Fig. 3-2. It is found from Tables 3-1 and 3-2 that the addition of SDS in the eluent improves the repeatability of the retention time of the analyte cations.

Table 3-1 Relative standard deviation (RSD) of retention time without SDS in the eluent

	RSD, % (*n*=6)
Analyte	Retention time
Li^+	4.5
Na^+	5.62
NH_4^+	6.22
K^+	6.9
Rb^+	7.13
Cs^+	7.84

Table 3-2 Summarized data for column efficiency, relative standard deviations (RSDs) of the retention times and peak height as well as limits of detection (LOD) obtained under the optimum operating condition as Fig. 3-2

	N max	RSD, % (*n*=6)		LOD (S/N=3)	
Analyte	Plates/m	Retention time	Peak height	mM	ppm
Li^+	13,700	0.46	2.21	0.357	2.5
Na^+	20,100	0.55	3.25	0.308	7.1
NH_4^+	8,400	0.52	2.74	0.101	1.8
K^+	25,000	0.45	2.92	0.512	20
Rb^+	12,000	0.39	2.08	0.796	68
Cs^+	15,200	0.58	3.11	0.603	80

3.4 Conclusions

A simple and direct capillary ion chromatographic method using C30 (100 × 0.32 mm. i.d.) stationary phase coated with SDS was developed. Li^+, Na^+, NH_4^+, K^+, Rb^+ and Cs^+ could be separated and detected by contactless conductivity detector by using 40 mM MSA and 0.25 mM SDS as the eluent, but unfortunately divalent cations could not be eluted under these conditions. In this case the concentration of the MSA in the mobile phase was kept constant at 40 mM. The reason for adding a low concentration of SDS to the eluent was to replenish some that are lost due to poor binding stability on the C30 surface. The addition of SDS in the eluent improved the repeatability of the retention time of the analyte cations. The contactless conductivity detector gave the better sensitivity and a more simple method than indirect UV detection where copper

sulfate is used as the co-additive in the eluent. Further improvement of LOD will be necessary for the determination of lower concentration of analyte cations.

3.5 References

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Chapter 4

Capillary ion chromatography with contactless conductivity detection for the separation of inorganic cations using polymer monolithic column

4.1 Introduction

During the last 15-20 years, there has been a trend to alleviate the column size in HPLC in order to take the advantages such as improved mass sensitivity, reduced consumption of packing material, mobile phases and sample amount and easiness of coupling to MS [1]. In some cases for example proteomics, the downsizing of sample made a significant problem. The increasing of demand for the throughput analysis with the high efficiencies encouraged development of high speed LC techniques and tools [2, 3]. The important thing to get a rapid and highly efficient separation is to reduce the column length with a smaller packed particle and also to increase the flow rate. Unfortunately, these conditions increased back pressure. To relieve the backpressure problem and get higher efficient separation, researchers started to develop monolith columns.

Monolithic columns have a great attention because easiness of modifications, improved chromatographic properties, fast mass transfer, easy control of permeability and no need of frits preparation. In 1989, Hjerten *et al.* firstly developed compressed soft polyacrylamide gels called “continuous bed” and used them to separate protein [4]. Another researchers reported rigid

macroporous organic polymer monolith and produced the different procedure and vision for the preparation [5]. There are two types of monolithic columns based on their components: organic and inorganic polymers. Silica based monolith belongs to inorganic polymers. Organic polymer monolithic materials can be classified into three general categories: polystyrene, methacrylate and acrylamide [6]. Methacrylate-based polymer becomes the most popular among the other organic polymer monolithic materials because of its advantages such as high stability in a broad pH range, fast and simple preparation, when using glycidyl methacrylate (GMA) as monomer, it is easy to modify because it has epoxy groups [7,8].

Nowadays, the sensitivity of detection techniques becomes one of the important research field on monolithic columns. UV absorption detection is most commonly preferred because of its low cost and wide applications [9]. Besides that, amperometry [10], mass spectrometry [11], chemiluminescence [12] are used for monolithic column detection.

Conductivity detection has been simple and universal detection technique in IC because it can detect many kind of ionized species with good sensitivity. There are two general types of conductivity detections: the metal electrodes can be directly contacted with liquid (galvanic detection) and separated from liquid with insulating film (contactless detection). Contactless conductivity detection has gained increasing attention in capillary liquid chromatography in recent years [8,13].

Karmarker *et al.* [14] analyzed the effect of different matrix formulation on mechanical properties of bis-glycidyl methacrylate (GMA) and poly(ethylene glycol) dimethacrylate (PEGDMA). Linda's group [15] reported methacrylate-based diol monolithic columns for separation of polar and non-polar compounds in capillary LC. Preparation and modification of

poly GMA/PEGDMA with diethylamine to separate inorganic anions was studied by Rahmah and co-workers [16]. In this work, GMA as a monomer, PEGDMA as a crosslinker and this polymer monolith reacted with Na_2SO_3 to introduce sulfonate groups in order to get cation-exchange monolithic columns inside 0.32 mm i.d. fused silica column. Strong cation-exchange monolith containing sulfonic groups were successfully synthesized from poly(GMA/PEGDMA) with methanol and decanol as porogenic solvents by *in situ* copolymerization. The synthesized monoliths were used to separate cations such as Li^+, Na^+, Rb^+. The effect of temperature condition, reaction time condition, and addition of organic solvent were studied.

4.2 Experimental

4.2.1 Apparatus

All experiments were assembled with 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 injector with an injection volume of 0.2 μL (Rheodyne, Cotati, CA, USA) as an injector, a 0.32 mm i.d.×100 mm microcolumn, and a Tracedec contactless conductivity detector (Istech, Strasshof, Austria). The flow-rate of the pump was kept at 3 μL/min. The data were acquired by a Chromatopac C-R7Ae plus data processor (Shimadzu, Kyoto, Japan).

4.2.2 Reagents and materials

3-(Trimethoxysilyl)propyl methacrylate (γ -MAPS), PEGDMA and 1-decanol were purchased from Tokyo Chemical Industry (Tokyo, Japan). GMA (97% pure), 2,2'-azobis(isobutyronitrile) (AIBN), methanol were obtained from Wako Pure Chemical Industries (Osaka, Japan). Purified water was produced in the laboratory by using a Millipore Simplicity UV system (Darmstadt, Germany). All the solutions in this study were prepared using the purified water.

4.2.3 Cation-exchange monolithic column preparation

The capillary column was washed with 1M NaOH, 1M HCl and purified water for 2 h, respectively. The inner wall of capillary was pretreated with γ -MAPS to make sure the anchoring the matrix of monolithic polymer. γ-MAPS in methanol were injected into the capillary column, and both ends of the capillary were sealed with teflon tube. The pretreatment was left to proceed in water bath at 60 ^{o}C for 24 h. Subsequently, the capillary was washed with acetone to flush out the residual reagent and dried with N_2 for 30 min.

The monolithic columns were prepared by *in situ* polymerization consisting of the monomer GMA, PEGDMA, the porogens of methanol and decanol. After sonicated for 5 min, AIBN were added to the monomer solutions (1% w/w corresponding to the total monomers), and purged with N_2 for 3 min. The pretreated capillary was filled with the monomer solutions and immediately sealed with teflon tube. Finally, the capillary was submerged in the water bath at 60

^{o}C for overnight. After polymerization, the capillary was washed with acetonitrile to remove the unreacted monomers and porogenic solvents present in the column.

The poly (GMA-PEGDMA) was flushed with 1M Na_2SO_3 in order to attach the sulfonate groups [5]. Then, the cation-exchange columns were rinsed with 5mM HNO_3 and water.

4.3 Results and Discussions

4.3.1 Column characterization

Cation-exchange monolith columns firstly were prepared from polymerization of GMA and PEGDMA with methanol and decanol as porogenic solvents. After that, poly(GMA-PEGDMA) was reacted with the Na_2SO_3 to introduce the sulfonate groups. The ratio between the monomer and the porogen phases will influence the flow-through pore size within a broad range, and the morphology of the monolith columns is depended on the composition of the porogen solvents, as well as on the composition of the monomer and crosslinker [17]. Figure 4-1 shows the SEM images of poly (GMA-PEGDMA) monolithic columns. In this monolith column, decanol was used as a porogen solvent to produce the throughpores in the polymer, while methanol was used to produce the mesopores, and could give the best flow-through characteristic [18]. The skeleton of monolith is approximately 2-3 μm.

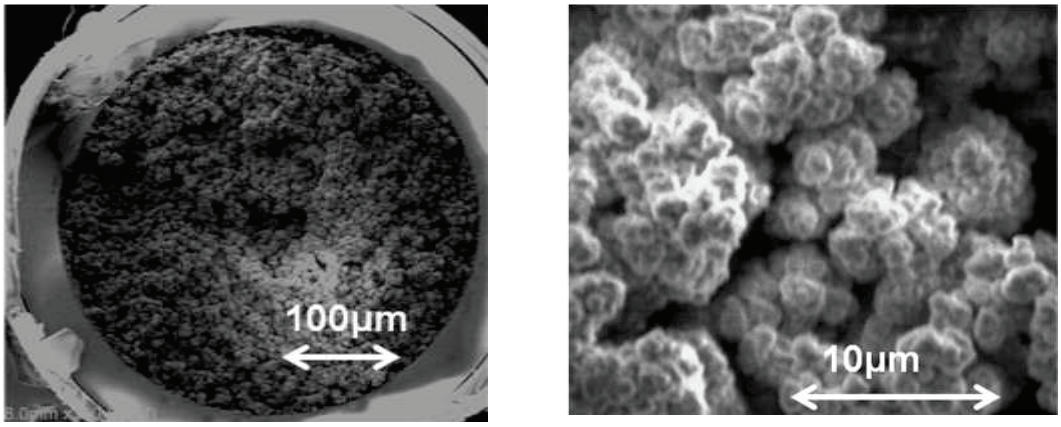

Fig. 4-1 The SEM images of poly (GMA-PEGDMA) monolithic columns.

4.3.2. Effect of temperature in modification

Temperature will take an important part in modification condition. In this work, we tried to modify at the temperature from 70-90 ^{o}C.

Table 4-1 The composition of polymerization of the column with the variation of temperature

Column	Monomer mixture		Porogen %(v/v)			%	1 M Na_2SO_3
	GMA	PEGDMA	MeOH	Decanol	(^{o}C/h)	Porogen	ml
A	12	20	4	64	80/8	68	0.5
B	12	20	4	64	90/8	68	0.5
C	12	20	4	64	100/8	68	0.5

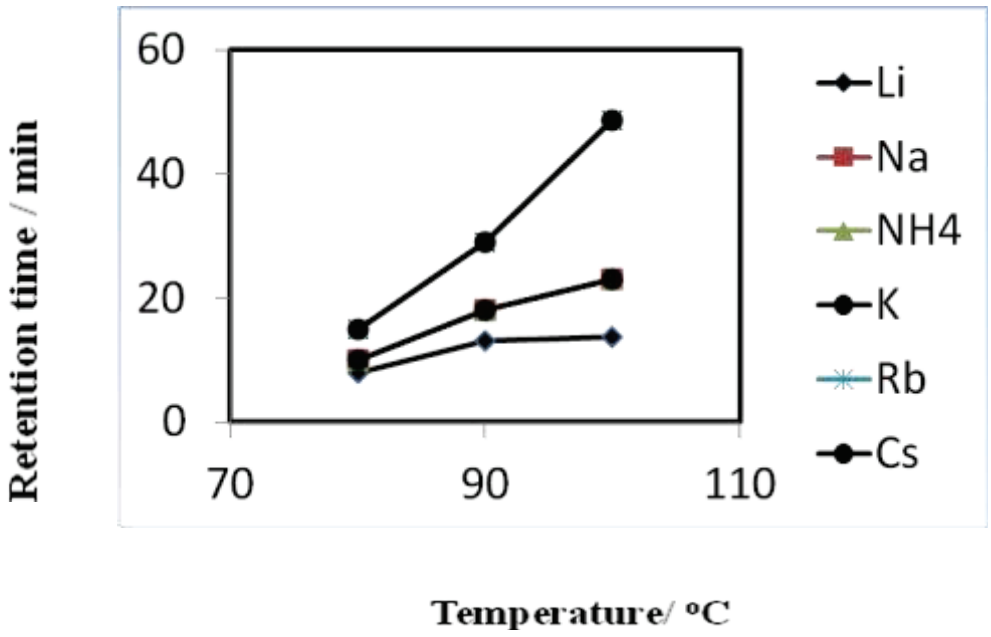

Fig. 4-2 The effect of temperature in modification with 1 M Na_2SO_3.

Figure 4-2 illustrates the retention time of analyte cations as a function of modification temperature of polymer monolith. It can be seen that the retention time of analyte cations increased with increasing temperature. By increasing the modification temperature, it could be expected more Na_2SO_3 is introduced on the polymer monolithic column and retained the analyte cations.

4.3.3. Effect of reaction time in modification

Beside temperature, the reaction time could be considered in modification of polymer monolithic column. We tried to modify for the reaction time from 8-16h.

Table 4-2 The polymerization composition of the column with the variation of reaction time

Column	Monomer mixture		Porogen %(v/v)			%	Na_2SO_3 1 M
	GMA	PEGDMA	MeOH	Decanol	(^oC/h)	Porogen	ml
A	12	20	4	64	80/8	68	0.5
B	12	20	4	64	80/12	68	0.5
C	12	20	4	64	80/16	68	0.5

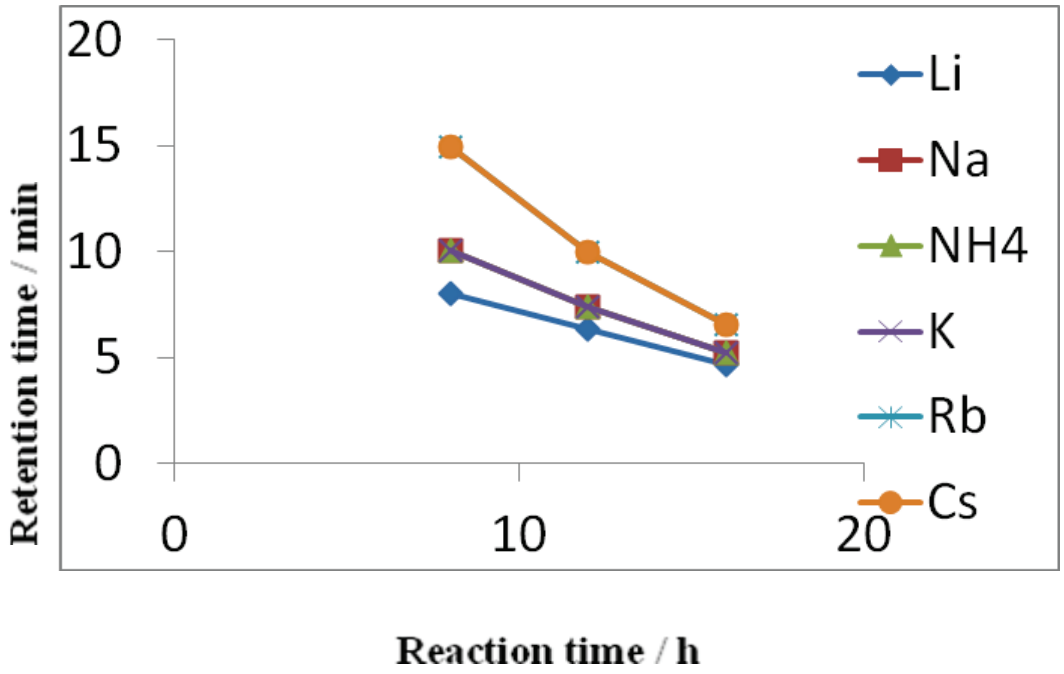

Fig. 4-3 The effect of reaction time in modification with 1 M Na_2SO_3.

Retention time of inorganic monovalent cations as a function of the reaction time for modification of polymer monolith column is shown in Figure 4-3. It can be seen that the shorter the reaction time, the longer is the retention time of analyte cations. Attaching Na_2SO_3 into the poly(GMA/PEGDMA) at 80 ^oC for 8h shows the best profile.

4.3.4 Effect of the organic solvent in the eluent

Figure 4-3 demonstrates the separation of the monovalent cations on the poly(GMA/PEGDMA) modification with 1 M Na_2SO_3. In some cases, adding methanol into the eluent can give the better selectivity of the performance in chromatographic system. From the chromatogram, it can be seen that methanol gives a better selectivity than acetonitrile. Unfortunately, in our work from the six monovalent cations were injected, only three cations could be separated which is lithium, sodium and rubidium.

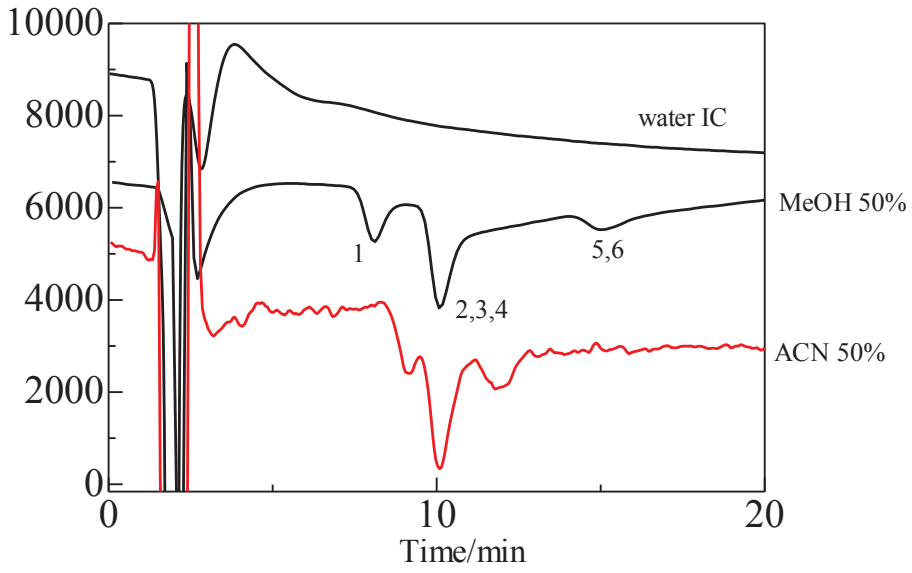

Fig 4-3 Separation of monovalent cations on poly(GMA/PEGDMA) modified with 1 M Na_2SO_3.

Column, poly(GMA/PEGDMA)/ 1 M Na_2SO_3, 100 × 0.32 mm i.d.; eluent, 5 mM MSA; flow-rate, 3.0 μL min^{-1}; concentration of analytes, analytes (concentration in 1 mM), 1=Li^+, 2=Na^+, 3=NH_4^+, 4=K^+, 5=Rb^+, 6=Cs^+; injection volume, 0.2 μL; detection, contactless conductivity detector.

4.4 Conclusions

Polymer-based strong cation-exchange monolithic stationary phases were prepared by thermal copolymerization of GMA as monomer, PEGDMA as crosslinker using binary porogenic solvents in the fused silica capillary with diameter of 0.32 mm. There are two step procedures to build up polymer monolithic stationary phases: synthesis of rigid polymer matrix (step 1) and sulfonation (step 2). Polymer monolithic GMA/PEGDMA modified with 1 M Na_2SO_3 (80 ^oC, 8H) and 68% porogen content could separate lithium, sodium and rubidium with the 5mM MSA in 50% methanol as eluent.

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Chapter 5

Preparation of one-pot synthesis AMPSA stationary phase for separation of inorganic cations in capillary ion chromatography

5.1 Introduction

Capillary ion chromatography (IC) is a promising chromatographic technique which can allow the separation of trace inorganic and organic ions. In 1983, Rokushika *et al.* [1] initiated the use of capillary tubes in their IC system and after that the alleviation of the column size in IC becomes a trend. The capillary IC has many advantages such as reduced consumption of packing material, mobile phase and also the sample amount, easiness of coupling with MS and improved mass sensitivity [2].

Various stationary phases have been used in IC since its development [3], including polymer-based [4] or silica-based [5]. Recently, a novel porous material known as monolithic column has been studied and applied in capillary IC. Polymer monolithic column has grown to become the interesting attention in capillary IC because of various advantages, such as wide selection of monomer with different functional groups, no need preparation of frits, simple polymerization procedure and wide application of pH values [6].

Zwitterionic ion chromatography has become a great attention group of stationary phase which can perform multi-selective retention mechanism. There are so many types of zwitterionic

ion chromatography such as stationary phase with covalently attached zwitterionic molecules [7-8], dynamically modified with zwitterionic molecules [9-10], modification of the ion-exchanger surface with the polymer of opposite charge (polysaccharides[11]or polyelectrolytes[12]). In 1951, Stach [13], firstly reported the synthesis of zwitterionic ion-exchange resin that contain both quarternary amino groups and sulfonic acid. 2-Acrylamido-2-methylpropanesulfonic acid is (AMPSA) is a multifunctional monomer that can form a dense network structure after polymerization because of its sulfonic acid functional group as strong cation exchanger at the end. Zakaria *et al.* prepared by in situ polymerization of butyl methacrylate (BMA), ethylene dimethacrylate, and AMPSA within fused-silica capillaries and also modified by using quarternary ammonium functionalized latex molecules for separation of anions [14]. Horiguchi *et al.* compared BMA-EDMA-MAA with BMA-EDMA-AMPSA monolithic column with the variation of percentages of ionic monomers in electrochromatography [15]. Gokaltun *et a.l* used AMPSA as monomer in monolithic column as a reference column and compared with 3-chloro-2-hydroxypropyl methacrylate (HPMA-Cl)/EDMA modification with $NaHSO_3$ for separation of alkylbenzenes, phenols and benzoic acids in capillary electrochromatography [16]. Another researcher also prepared both strong and weak cation exchange stationary phase using photografting of AMPSA and acrylic acid for separation of basic mixture and digest of protein [17].

In this research, we synthesized one-pot the capillary monolithic column by using AMPSA as monomer and EDMA as crosslinker. AMPSA has a strong cation exchanger fuctional groups such sulfonic acid. We expected to use AMPSA as monomer and EDMA as crosslinker for separation of inorganic cations by using methanesulfonic acid and methanol as eluent with

contactless conductivity detector. The composition of polymerization mixture, effect of addition of organic solvent and the separation ability were investigated.

5.2 Experimental

5.2.1 Apparatus

All experiments were assembled with 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 injector with an injection volume of 0.2 μL (Rheodyne, Cotati, CA, USA) as an injector, a 0.32 mm i.d.×100 mm microcolumn, and a Tracedec contactless conductivity detector (Istech, Strasshof, Austria). The flow-rate of the pump was kept at 3 μL/min. The data were acquired by a CDS data processor (LASOFT, Chiba, Japan).

5.2.2 Reagents and materials

3-(Trimethoxysilyl)propyl methacrylate (γ-MAPS), AMPSA and EDMA were purchased from Tokyo Chemical Industry (Tokyo, Japan)., 2,2'-azobis(isobutyronitrile) (AIBN), methanol, 1, 4-butanediol, 1-propanol, acetone were obtained from Wako Pure Chemical Industries (Osaka, Japan). Purified water was produced in the laboratory by using a Millipore Simplicity UV system (Darmstadt, Germany). All the solutions in this study were prepared using the purified water.

5.2.3 AMPSA monolithic column preparation

The capillary column was washed with 1M NaOH, 1M HCl and purified water for 30 min, respectively. The inner wall of capillary was pretreated withγ-MAPS to make sure the anchoring the matrix of monolithic polymer. γ-MAPS in acetone were injected into the capillary column, and both ends of the capillary were sealed with teflon tube. The pretreatment was left to proceed in water bath at 60 ^{o}C for 24 h. Subsequently, the capillary was washed with acetone to flush out the residual reagent and dried with N_2 for 30 min.

The monolithic columns were prepared by *in situ* polymerization consisting of the monomer AMPSA, EDMA, the porogens of 1-propanol, 1,4 butanediol and methanol. After sonicated for 5 min, AIBN was added to the monomer solutions (1% w/w corresponding to the total monomers), and purged with N_2 for 3 min. The pretreated capillary was filled with the monomer solutions and immediately sealed with teflon tube. Finally, the capillary was submerged in the water bath at 60 ^{o}C for overnight. After polymerization, the capillary was washed with water and 10 mM HNO_3 to remove the unreacted monomers and porogenic solvents present in the column.

5.3 Results and Discussions

5.3.1. Effect of eluent variation

In this research, we used contactless conductivity as detector and weak organic acids are usually used as eluent for separation and determination inorganic cations in non-suppressed ion chromatography. Some organic acids such as tartaric acid, 5-sulfosalicylic acid and methanesulfonic acid (MSA) have been used for separation of inorganic cations. We intended that the increasing of concentatration decreased the retention time, but with the same electric

charge showed similar retention times. The composition of monolithic column was 16% v/v AMPSA, 16% v/v EDMA, 64% v/v decanol and 4% methanol, and prepared at 60 ^{o}C for 24h. The performance of our chromatographic properties appears to be affected by the eluent that we used. Figure 5-1 shows the separation of inorganic cations on poly(AMPSA/EDMA) with various eluent. The MSA gave better selectivity compared with other organic acids examined in this work, MSA was selected as the eluent for separation of cations in the next experiments.

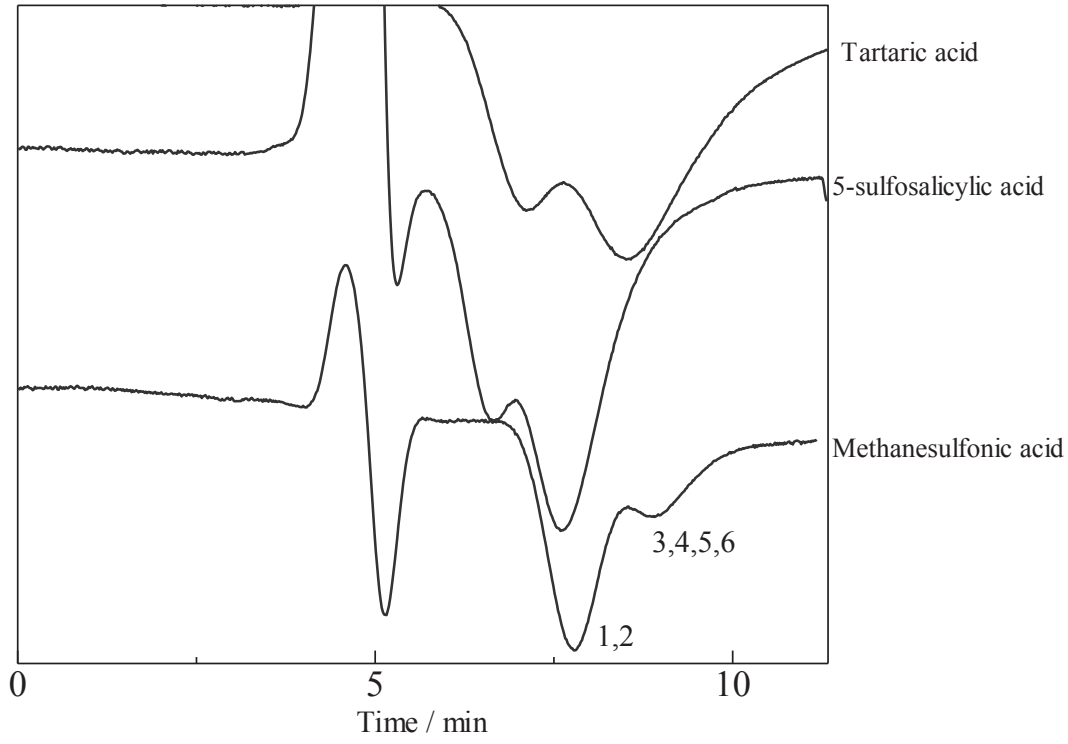

Fig 5-1 Separation of inorganic cations on poly(AMPSA/EDMA) with variation of eluent

Column, poly(AMPSA/EDMA) 100 × 0.32 mm i.d.; flow-rate, 3.0 μL min^{-1}; concentration of analytes, analytes (concentration in 1 mM), 1=Li^+, 2=Na^+, 3=NH_4^+, 4=K^+, 5=Rb^+, 6=Cs^+; injection volume, 0.2 μL; detection, contactless conductivity detector

5.3.2 Effect of polymerization variation (monomer with porogen)

The polymerization of AMPSA and EDMA with 1-propanol, 1,4-butanediol and methanol as porogenic solvents was carried out in order to produce cation-exchange monolith columns in one step. The various compositions of polymerization mixture in this study are shown in Table 5-1. It is known that even the little changes of the composition of the polymerization mixtures affect the performance of the AMPSA monolithic columns. In this research, we tried to vary the concentration of AMPSA from 15 to 25 % v/v by using 5mM MSA as eluent. Figure 5-1 displays separation of inorganic cations on poly(AMPSA/EDMA). From the figure it is found that the retention time between the column A, B, C is almost the same, but the better shape peaks are observed for the column A, and it also can separate monovalent cations (lithium, ammonium, rubidium) and divalent cation (calcium/ magnesium). Unfortunately, the column B and C could separate monovalent and divalent cations.

Table 5-1 Polymerization of the column with the various monomer and porogen compositions

Column	AMPSA % v/v	EDMA % v/v	1-Propanol % v/v	1,4-butanediol % v/v	MeOH % v/v	Temp./Tem	Initiator (mg)
A	15	25	40	15	5	60^{o}C/24H	4
B	20	20	40	15	5	60^{o}C/24H	4
C	25	15	40	15	5	60^{o}C/24H	4

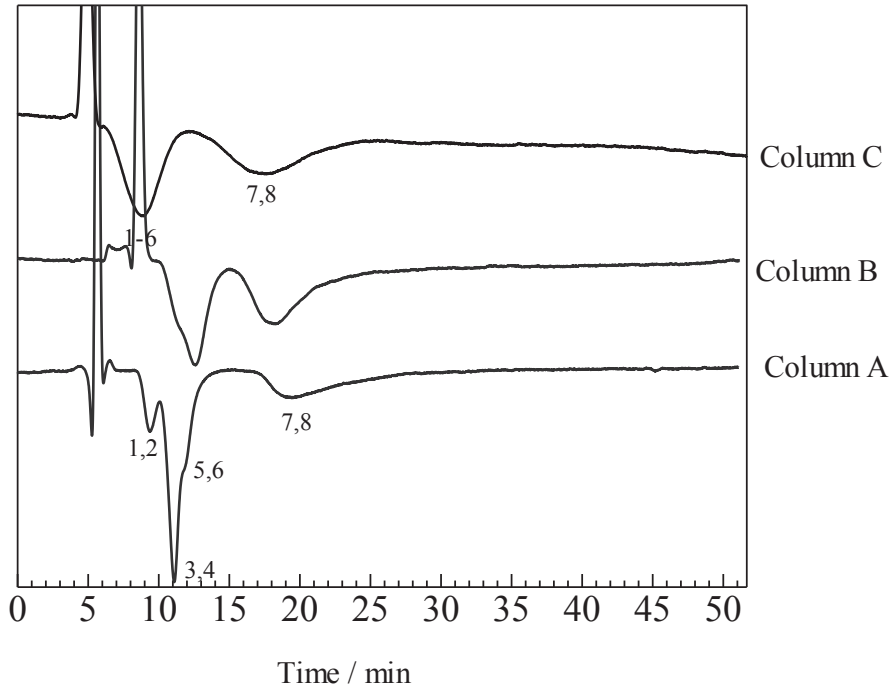

Fig 5-2 Separation of inorganic cations on poly(AMPSA/EDMA)

Column, poly(AMPSA/EDMA) 100 × 0.32 mm i.d.; eluent, 5mM MSA; flow-rate, 3.0 μL min^{-1}; concentration of analytes, 1 mM each; analytes, 1=Li^+, 2=Na^+, 3=NH_4^+, 4=K^+, 5=Rb^+, 6=Cs^+, 7=Ca^{2+}, 8=Mg^{2+}; injection volume, 0.2 μL; detection, contactless conductivity detector

5.3.3. Effect of organic solvent and crown ether addition in the eluent

Beside the eluent and the composition of polymerization mixture, addition of another organic solvents could be considered in AMPSA monolithic columns. Some of the organic solvents such as methanol, acetonitrile can effectively improve the resolutions, peak shape and retention times [18]. So, in our research, we tried to investigate the effect of the organic solvents addition in the eluent. Figure 5-3 shows the separation of inorganic cations on poly (AMPSA/EDMA). The

retention times of inorganic cations increased by adding 50% methanol, 50 %acetonitrile and 1mM crown ether but, the selectivity and peak shape for analyte cations were not improved. It can be expected adding the organic solvents and crown ether in the eluent can reduce the MSA ability to elute the analyte cations.

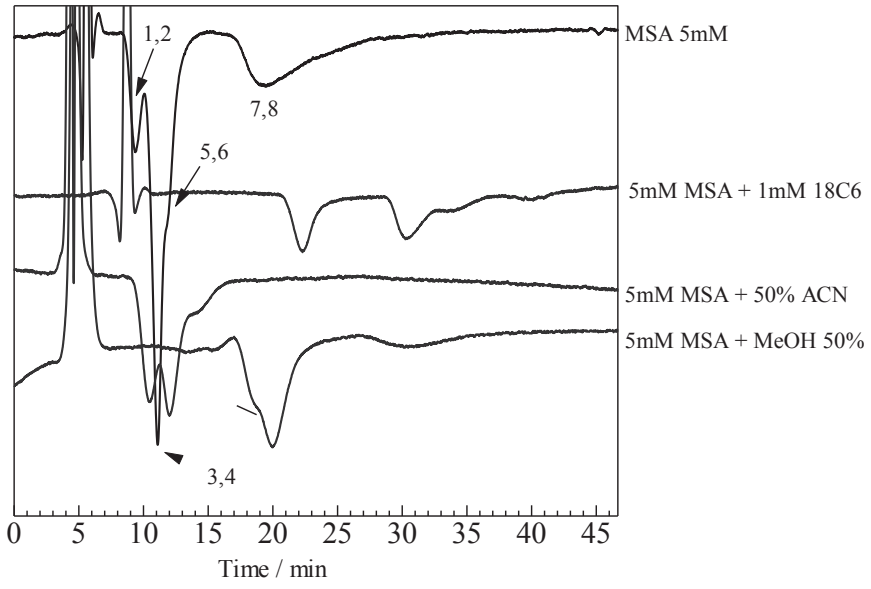

Fig 5-3 Separation of inorganic cations on poly(AMPSA/EDMA) with organic solvents.

Column, poly(AMPSA/EDMA) 100 × 0.32 mm i.d.; flow-rate, 3.0 μL min^{-1}; concentration of analytes, 1 mM each; analytes, 1=Li^+, 2=Na^+, 3=NH_4^+, 4=K^+, 5=Rb^+, 6=Cs^+, 7=Ca^{2+} , 8=Mg^{2+}; injection volume, 0.2 μL; detection, contactless conductivity detector.

5.4 Conclusions

One-pot synthesis strong cation exchange monolithic stationary phases were prepared by thermal copolymerization of AMPSA as monomer, EDMA as crosslinker using 1-propanol, 1,4-butanediol and methanol as porogenic solvents in the fused silica capillary with diameter of 0.32

mm. Polymer monolithic AMPSA/EDMA prepared at 60 ^{o}C and for 24 h could separate monovalent and divalent cations, with 5mM MSA as eluent. However, this research could not fully separate each monovalent cation and also each divalent cation.

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Chapter 6

Conclusions and Future perspective

6.1 Conclusions

Monovalent cations were separated with good selectivity on the C30 stationary phase by using PFOS as the eluent additive, where copper sulfate as the co-additive in the eluent allowed the indirect detection of the cations. PFBS as the eluent additive provided poor selectivity although monovalent and divalent cations were eluted under the same eluent conditions. It is expected that the use of conductivity detection will simplify the eluent conditions.

A simple and direct capillary ion chromatographic method using C30 (100 × 0.32 mm. i.d.) stationary phase coated with SDS was developed. Monovalent cations could be separated by contactless conductivity detector by using 40 mM MSA and 0.25 mM SDS as the eluent, but unfortunately divalent cations could not be eluted under these conditions. In this case the concentration of the MSA in the mobile phase was kept constant at 40 mM. The reason for adding a low concentration of SDS to the eluent was to replenish some that are lost due to poor binding stability on the C30 surface. The addition of SDS in the eluent improved the repeatability of the retention time of the analyte cations. The contactless conductivity detector gave the better sensitivity and a more simple method than indirect UV detection where copper

sulfate is used as the co-additive in the eluent. Further improvement of LOD will be necessary for the determination of lower concentration of analyte cations.

Polymer-based strong cation-exchange monolithic stationary phases were prepared by thermal copolymerization of GMA as monomer and PEGDMA as crosslinker using binary porogenic solvents in the fused silica capillary with diameter 0.32 mm. There are two step procedures to build up polymer monolithic stationary phase: synthesis of rigid polymer matrix (step 1) and sulfonation (step 2). Polymer monolithic GMA/PEGDMA modification with 1M Na_2SO_3 (80 ^oC, 8h) and porogen content 68% could separate lithium, sodium and rubidium with 5mM MSA in 50% methanol as eluent.

One-pot synthesis strong cation exchange monolithic stationary phases were prepared by thermal copolymerization of AMPSA as monomer, EDMA as crosslinker using 1-propanol, 1,4-butanediol and methanol as porogenic solvents in the fused silica capillary with diameter of 0.32 mm. Polymer monolithic AMPSA/EDMA prepared at 60 ^oC and for 24 h could separate monovalent and divalent cations, with the 5mM MSA as eluent. However, this research could not fully separate each monovalent cation and also each divalent cation.

6.2 Future perspective

Since its introduction in 1975 [1], ion chromatography (IC) has been utilized in most areas of analytical chemistry and has become a versatile and powerful technique for the analysis of a large number of ions. Over three last decades, there has been much discussion focusing the promising capillary ion chromatography. In order to get the high separation efficiency and high resolution of HPLC, capillary LC is one of the interesting methods to separate the inorganic and organic ions. The development of ion chromatography allows the determination of ionic

contaminants in samples with very low detection limits and expands the range of determined analytes.

As an another alternative to the micro-packed capillary IC, monolithic polymer capillary columns have gained much attention in order to get higher permeability, so we can get the fast separation of inorganic cations by using a polymer monolithic column GMA/PEGDMA and as described in the Chapter 4. Optimization of the composition of polymerization mixture GMA/PEGDMA still remains a challenging task , which at least can separate the monovalent cations. Chapter 5 described one-pot synthesis of the polymer monolithic column especially zwitterionic compound for separation of inorganic cations. Zwitterionic ion chromatography has a great attention group of stationary phase which can perform multi-selective retention mechanism. There are so many types of zwitterionic ion chromatography with stationary phases covalently attached with zwitterionic molecules [2-3] and dynamically modified with zwitterionic molecules [4-5]. One-pot synthesis of polymer monolithic column could serve a candidate for wide range of applications and high permeability. The exploration of other one-pot synthesis of polymer monolithic stationary phase especially zwitterionic compound in the simultaneous separation of cations and anions is also worthwhile.

Besides the novelty of the new technologies for the development of stationary phase and the eluent-induced stationary phase for separation of ions, it should be concerned to integrate the capillary LC with another method such as spectrometric detection. The most common is LC-MS; LC combined with diode-array UV absorbance.

6.3 References

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List of publications

1. Femi Earnestly, Lee Wah Lim, Toyohide Takeuchi, Separation of inorganic cations on a hydrophobic stationary phase using perfluoroalkanesulfonic acid as the eluent additive in ion chromatography, *Int. J. Chem.*, **4** (2012) 466-471. (Described in Chapter 2)
2. Femi Earnestly, Lee Wah Lim, Toyohide Takeuchi, Eluent induced separation of inorganic cations in capillary liquid chromatography with contactless conductivity detector, *Chromatographia* **77** (2014) 1539-1544. (Described in Chapter 3)

List of presentations

1. Femi Earnestly, Lee Wah Lim, Toyohide Takeuchi

   "Eluent induced separation of inorganic cations in capillary liquid chromatography", The 7th Chonnam National University-Faculty of Engineering Gifu University Joint Symposium, June 26, 2012 (Poster Presentation)

2. Femi Earnestly, Lee Wah Lim, Toyohide Takeuchi

   "Eluent induced separation of inorganic cations in capillary liquid chromatography", The 6th Asia-Pacific Symposium on Ion Analysis, Padang, Indonesia, November 26-28, 2012 (Oral Presentation)

3. Femi Earnestly, Lee Wah Lim, Toyohide Takeuchi

   Separation of inorganic cations using surfactant coated C30 stationary-phase in capillary liquid chromatography by contactless conductivity detector", The 62nd Annual Meeting of The Japan Society for Analytical Chemistry, Osaka, September, 10-12, 2013 (Oral Presentation)

4. Femi Earnestly, Lee Wah Lim, Toyohide Takeuchi

   "Capillary Ion Chromatography with Contactless Conductivity Detection for the Separation of Inorganic Cations using Polymer Monolithic Column", The 7th Asia-Pacific Symposium on Ion Analysis, Jeju Island, Korea, November, 3-6, 2013 (Oral presentation)

5. Femi Earnestly, Lee Wah Lim, Toyohide Takeuchi

   "Separation of inorganic cations using surfactant coated C30 stationary-phase in capillary liquid chromatography by contactless conductivity detector", The 30th Ion Chromatography Symposium, Aichi Nagakute City, November 28-29, 2013 (Oral Presentation)

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