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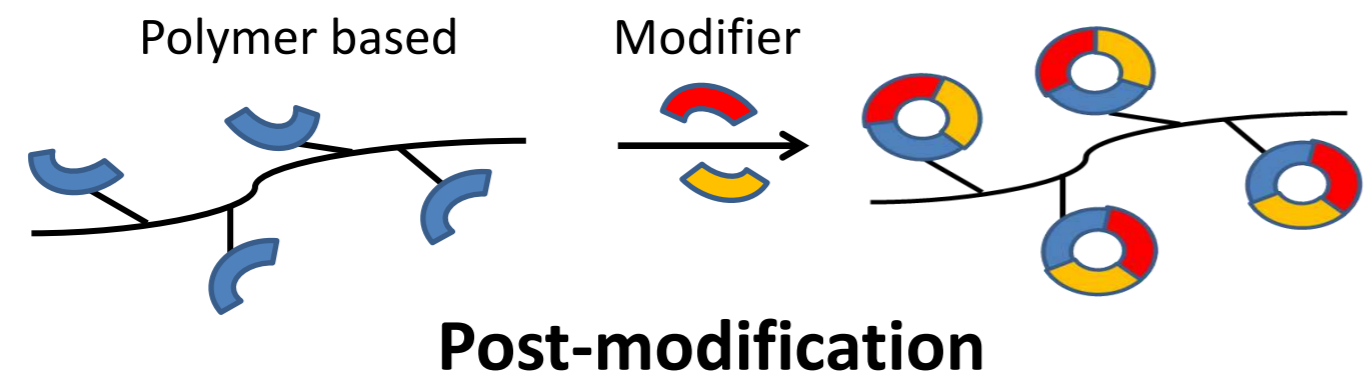


Preparation of Sulfonate Monolithic Column for Rapid and Simultaneous Separation of Inorganic Cations in Capillary Ion Chromatography

Introduction

Strong Cation Exchange monolithic column

- It has been widely applied for separation of proteins, peptides and inorganic cations.
- It by in-situ preparation of polymer-based organic and silica-based inorganic monoliths column by versatile post-modifications and in-situ chemical reactions.



One-pot approach

- It was introduced as a new strategy for more effective and sophisticated way in preparation of the monolithic column for achieving various target separations.
- It was due to eliminate the consuming of time for post-modifications.



OBJECTIVE

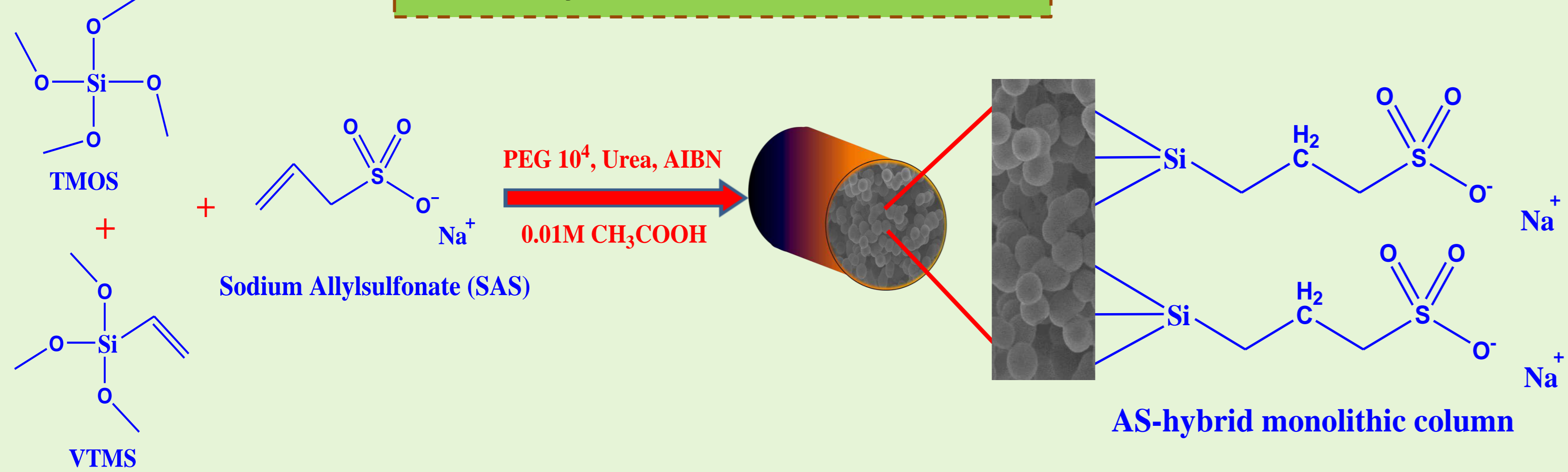
SCX-Hybrid
Polymer one-pot

Proteome analysis,
proteins, peptides

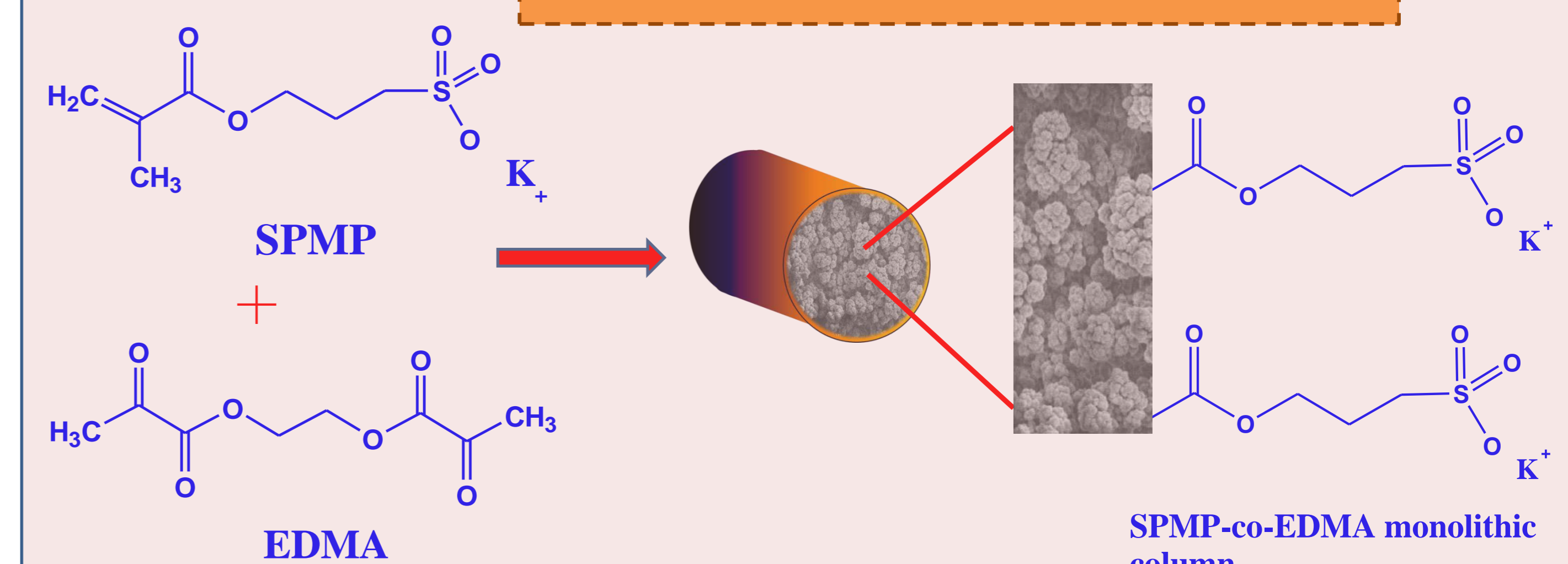
??? IEC

*IEC : Ion Exchange Chromatography

AS-hybrid monolithic column

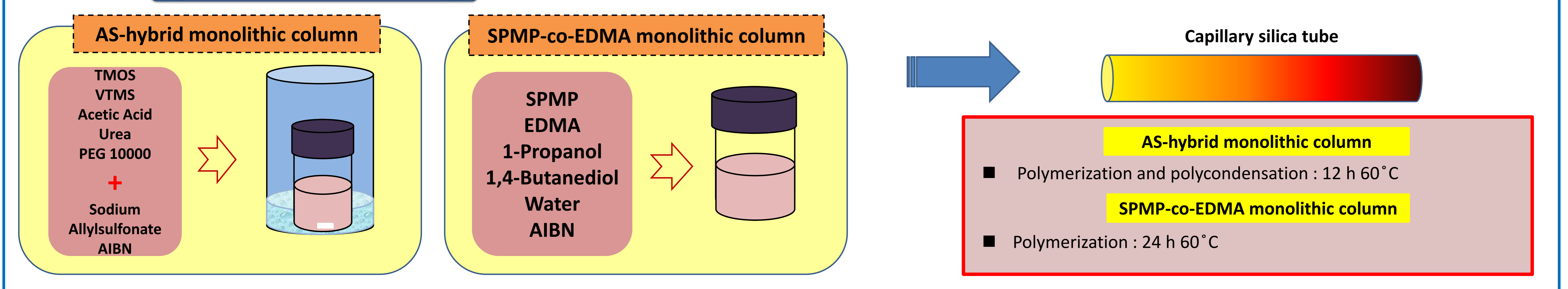


SPMP-co-EDMA monolithic column



AS-hybrid monolithic column prepared and SPMP-co-EDMA monolithic column by a single-step thermal-treatment one-pot manner.

Methods



Results

Table 1. Preparation of AS-Hybrid monolithic column

Column	SAS ^a (mg)	Time (h)	Temp (°C)	Back Pressure ^b (Mpa)	<i>k</i> permeability 10^{-10} (m ²)	<i>N</i> _{max} plates/m
A	35	9	60	0.2	11	6107
B	35	12	60	0.2	11	19017
C	35	18	60	0.5	4.4	4214
D	35	24	60	0.6	3.3	12707
E	35	36	60	0.8	2.6	8603
F	35	12	50	0.2	11	15869
G	35	12	70	0.3	7.3	19001
H	35	12	80	0.4	5.5	15636
I	30	12	60	0.2	11	8795
J	40	12	60	0.2	11	14100
K	45	12	60	0.2	11	14220
L	50	12	60	0.2	11	14618

^aSodium Allylsulfonate (SAS) as organic monomer
^bSystem operated with CuSO₄ at flow rate 2 μL/min

Table 2. Preparation of SPMP-co-EDMA monolithic column

Column	Monomer ^a % (v/v)	Crosslinker ^b % (v/v)	Porogen ^c % (v/v)	Mixture ratio ^d	Polymerization condition ^e	CEC meqiv/mL
SPMP1	7.5	2.5	90	10/90	60 °C, 24 h	0.50
SPMP2	15	5	80	20/80	60 °C, 24 h	0.52
SPMP3	22.5	7.5	70	30/70	60 °C, 24 h	0.58
SPMP4	30	10	60	40/60	60 °C, 24 h	0.69
SPMP5	30	10	60	40/60	50 °C, 24 h	0.63
SPMP6	30	10	60	40/60	70 °C, 24 h	0.84
SPMP7	30	10	60	40/60	80 °C, 24 h	0.81
SPMP8	5	35	60	40/61	70 °C, 24 h	0.42
SPMP9	20	20	60	40/62	70 °C, 24 h	0.52
SPMP10	35	5	60	40/63	70 °C, 24 h	0.85
SPMP11	39	1	60	40/64	70 °C, 24 h	0.58

^a 3-sulfopropyl methacrylate potassium salt (SPMP) was used as functional monomer.
^b Ethylene dimethacrylate (EDMA) was used as cross-linker.
^c 1-propanol, 1,4-butanediol and water were used as porogen.
^d The volume ratio of monomer + cross-linker to porogen.
^e 60 °C was the polymerization temperature and kept for 24 h.

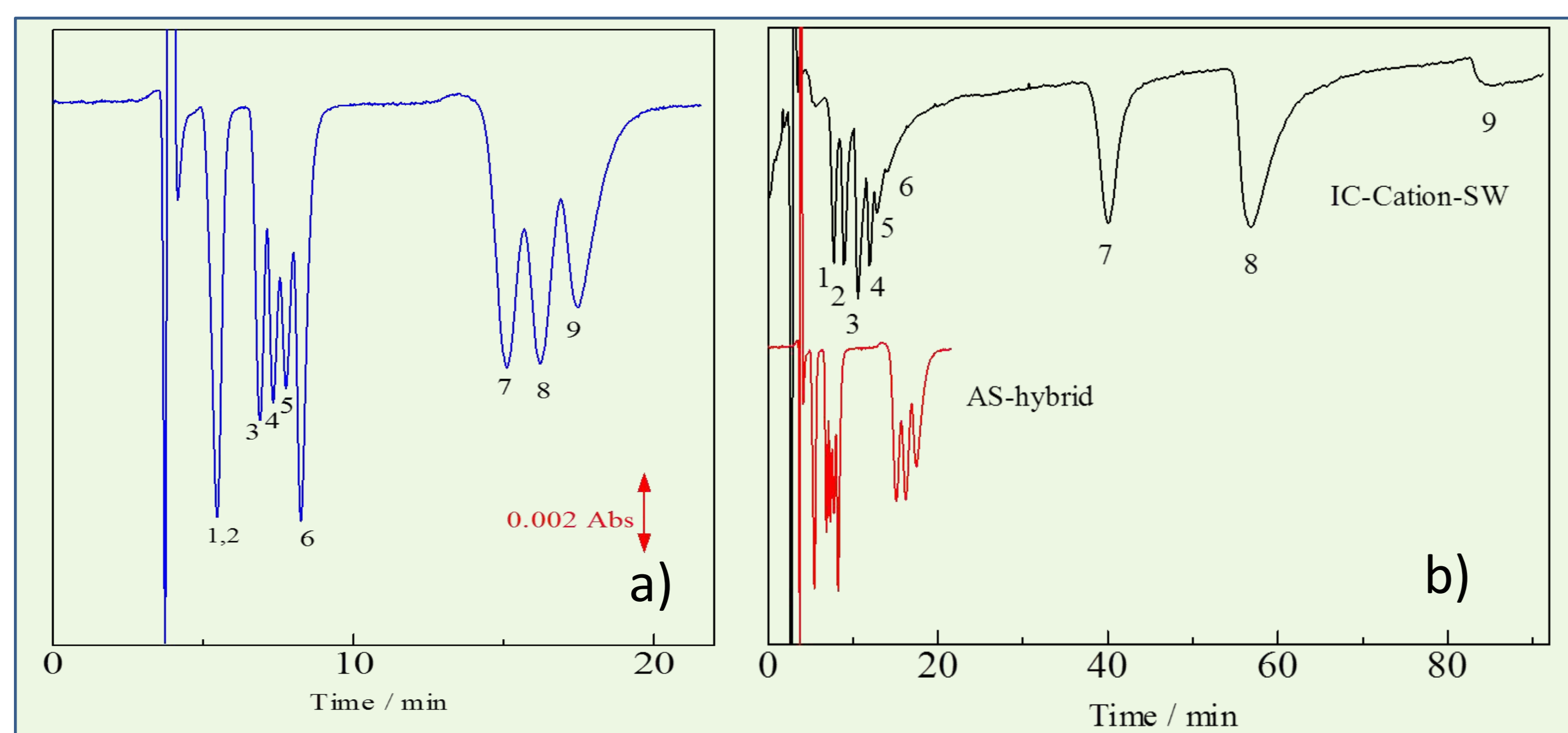
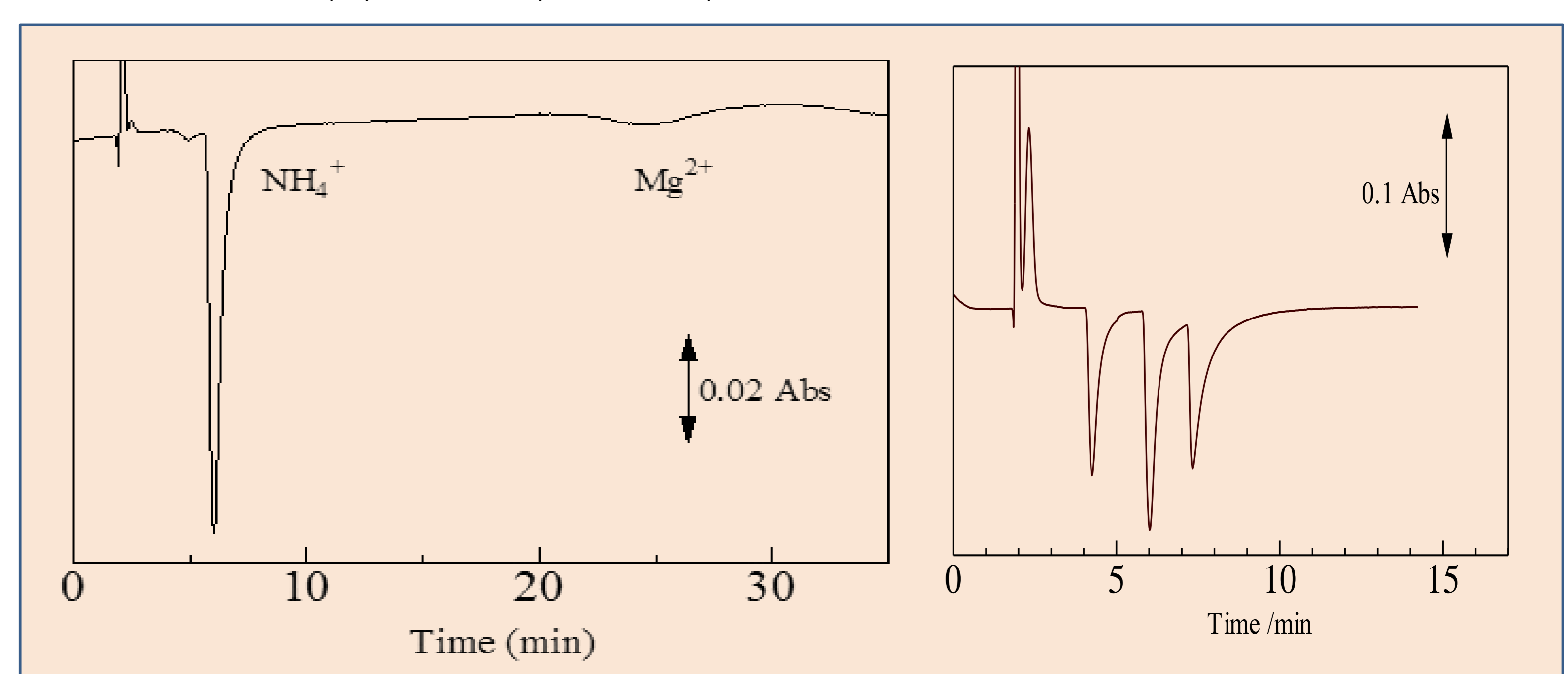
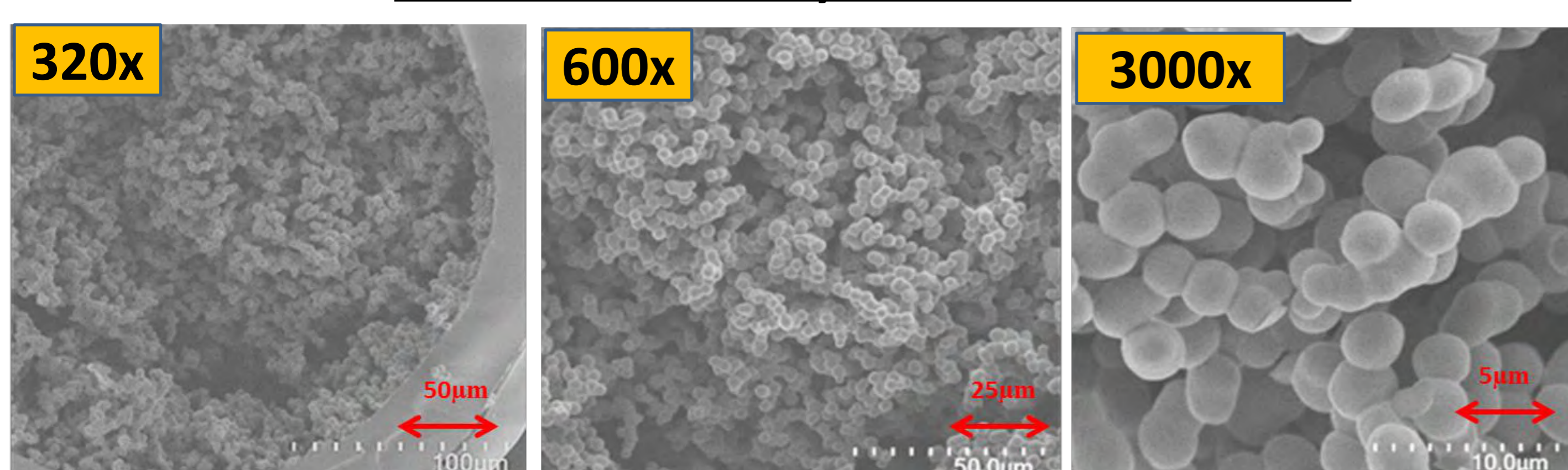


Fig.1 a) AS-hybrid column and b) column of AS-hybrid and IC-cation-SW (100 x 0.32 mm i.d.); Analytes, 1 mM of each Li⁺ (1), Na⁺ (2), NH₄⁺ (3), K⁺ (4), Rb⁺ (5), Cs⁺ (6), Mg²⁺ (7), Ca²⁺ (8), and Sr²⁺ (9); Eluent, 5 mM of CuSO₄; Flow rate 2 μL/min; UV detection, 210 nm wavelength; 0.2 μL injection vol.



Column, SPMP-co-EDMA monolithic column(100 x 0.32 mm i.d.); Analytes, 1 mM of a) NH₄⁺ and Mg²⁺ and b) Na⁺, NH₄⁺, and K⁺ Eluent, 5 mM of CuSO₄; Flow rate 4 μL/min; UV detection, 210 nm wavelength; 0.2 μL injection vol.

SEM Photo of AS-Hybrid monolithic column



Permeability (K) = 11×10^{-12} m²; The BET surface area = 164 m²/g; A narrow size distribution was approximately 1 nm with volume = 0.5 cm³/g

SEM Photo of SPMP-co-EDMA monolithic column

Conclusions

- An AS-hybrid and SPMP-co-EDMA monolithic capillary column with strong cation-exchange moieties was successfully fabricated for the simultaneous separation of common inorganic cations by **single-step thermal-treatment one-pot manner**.
- A novel application for rapid and simultaneous determination of both common monovalent and divalent inorganic cations in ion-exchange chromatography mode was found.



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

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ABSTRACT

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

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1. Introduction

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

Polymer monolithic stationary phases for ion exchange chromatography (IEC) were fabricated and the separation efficiency was investigated in this study. The fabrication of the polymer

monolith usually involves a reactive moiety of the monolith; in this case epoxy group was used, which is then directly modified with functional groups that contain ion-exchange property, such as amino groups [6].

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

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

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Table 1
Various polymerization conditions.

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

* Not applicable.

^a Glycidyl methacrylate (GMA) was always used as a monomer.

^b Ethylene dimethacrylate (EDMA) was used as a cross-linker.

^c Three kind of alcohols i.e. ethanol, 1,4-butanediol and decanol were used as porogen.

^d The volume ratio of monomer+cross-linker to porogen.

^e 1,4-Dioxane and pyridine were used as the solvents of tosylated-PEG during the modification.

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

2. Experimental

2.1. Chemicals

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

2.2. Preparation of monolithic column

The fused silica capillary tube (0.320 mm i.d. \times 0.450 mm o.d.) was purchased from GL Sciences (Tokyo, Japan). Fused silica capillary tube was washed using 1 M NaOH solution, deionized water and 1 M HCl in sequence. 30% (v/v) of γ -MAPS in acetone was used for providing methacrylate groups on the inner wall surface of the capillary tube; the tube was sealed at both ends and thermally treated in a water bath at 60 °C for 24 h. Thereafter, the

capillary tube was washed with acetone and dried using nitrogen gas for 30 min. As shown in Table 1, mixture solutions of monomer, cross-linker and porogen were prepared at various compositions. Then, 0.2 mL of the solution was completely mixed with 2 mg of AIBN, i.e. the polymerization initiator. The mixture solution was then subjected to ultrasonic vibration for 5 min before it was injected into the pretreated capillary tube. Thermal polymerization was carried out in the water bath at 60 °C for 24 h. The capillary tube was rinsed with methanol thoroughly after polymerization to remove unreacted reagent and porogenic solvents. The morphology of the monolith was examined by using scanning electron microscopy (SEM; S-4800, Hitachi, Tokyo, Japan).

2.3. Modification of glycidyl methacrylate base

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

2.4. Capillary liquid chromatography

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

3. Results and discussion

3.1. PEG-monolith preparation

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

great importance in production site. Generally, the performance of the monolith column was influenced by ratio of monomer to porogen. Hence, investigated ratio of monomer to porogen is necessary for achieving the best methacrylate base before continuing modification step. Firstly, the ratio of monomer to porogen was mainly investigated by keeping the cross-linker content. The resolution between two anions, i.e., IO_3^- and BrO_3^- is shown in Table 2. The monomer/porogen ratio is 20/80 has the best separation profile of anions with resolution approximately 1.15–1.77. When the monomer content was decreased to 10/90, the resolution value could not be calculated because all the anions appeared at the same retention time. Conversely, when the monomer content was increased up to 30/70 and 40/60, the separation of anions was deteriorated and some of the anion peaks were overlapped each other; it was evidenced by resolution values of 1.00–1.36 and 0.85–1.16, respectively. Less amount of porogen also can cause the tight or fully-packed structure of the monolith in the capillary tube. The morphology of the monolith surface was also observed by using SEM, as shown in Fig. 1. Theoretically, by increasing the ratio of porogen, the through-pores will also be increased; on the other hand, when the ratio of the porogen is less, the surface of the monolith will become fully

packed and will thus increase the back pressure. From the four SEM photos, showing the morphology of monoliths with the monomer/porogen ratio is 40/60, 30/70, 20/80 and 10/90, it is clearly shown that the monoliths were attached tightly to the inner-wall of the silica capillary tubes, and the estimation ranges of the monolith skeleton are 1–1.5, 2–2.5, 3–4 and 3.5–4 μm , respectively. The reference monolith M2 was chosen as the starting point to continue preparation of the PEG monolith.

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

Table 2

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

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

^a The volume ratio of monomer to porogen.

^b The resolution between IO_3^- and BrO_3^- .

3.2. Effect of modification conditions

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

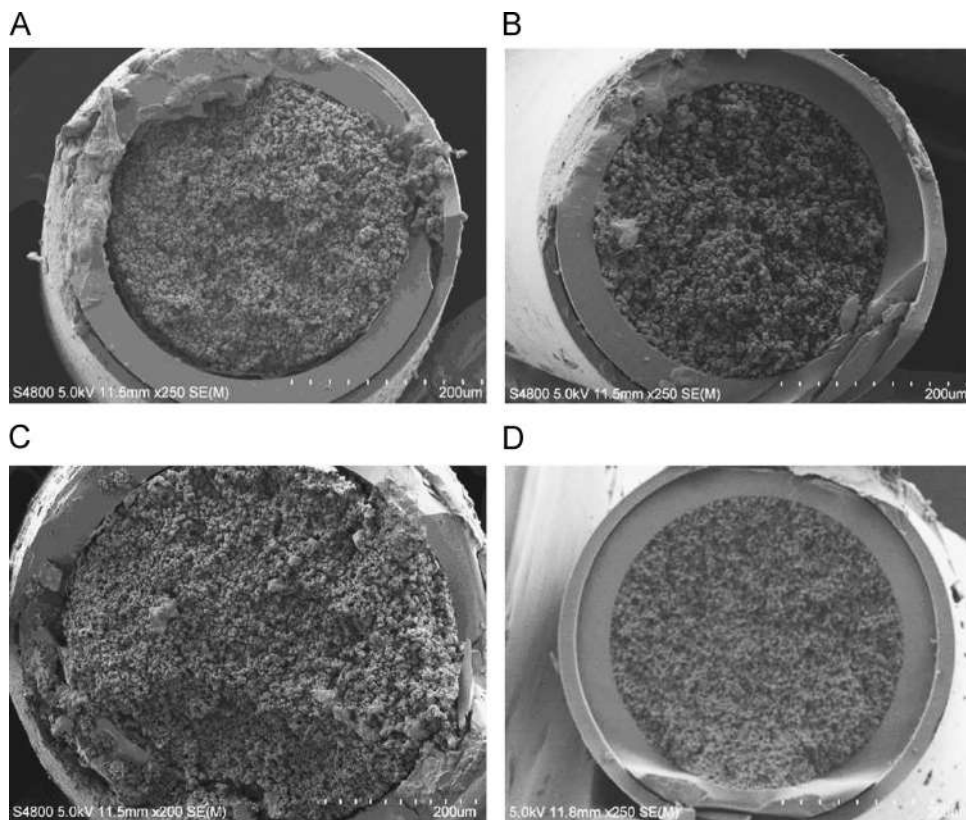


Fig. 1. Scanning Electron Microscope images of PEG-monolithic column. Monomer:porogen is 40/60 (A), 30/70 (B), 20/80 (C) and 10/90 (D).

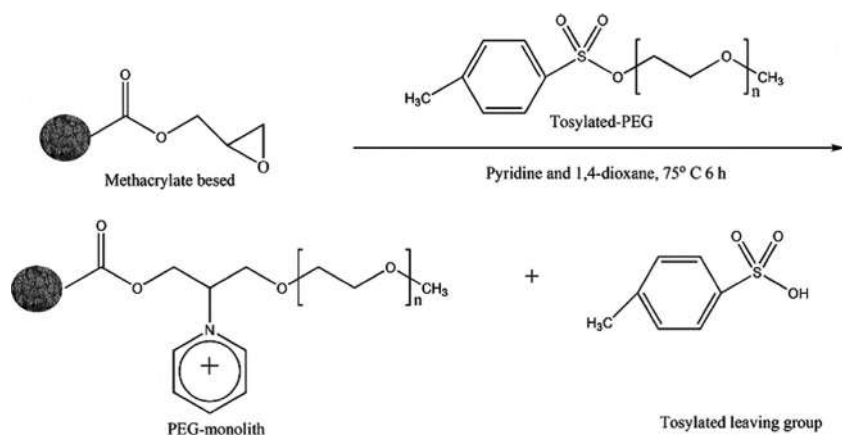


Fig. 2. Schematic diagram of the expected reaction for the preparation of PEG-monolithic column.

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

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

3.3. Optimization of eluents

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

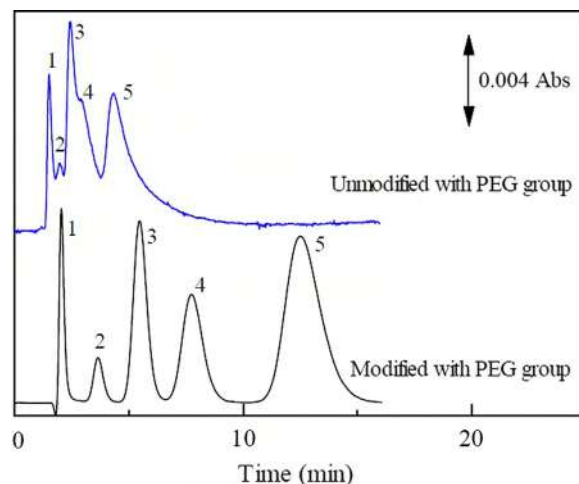


Fig. 3. Separation of inorganic anions on the stationary phase with and without PEG groups. Operating conditions: Analytes, 1 mM of each iodate (1), bromate (2), nitrite (3), bromide (4) and nitrate (5); PEG-monolith M2 column (100 \times 0.32 mm i.d.); 100 mM of sodium chloride eluent; 4 $\mu\text{L}/\text{min}$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

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

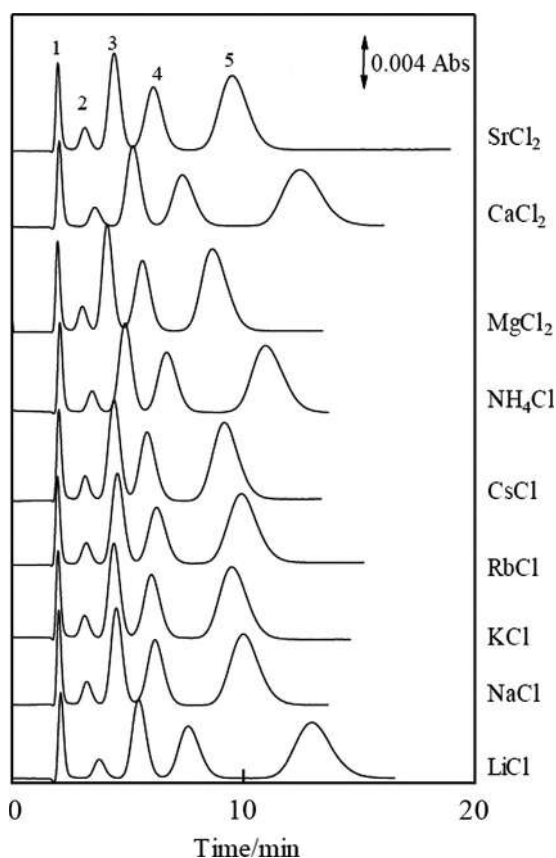


Fig. 4. Chromatograms of inorganic anions with various chloride eluents. Analytes, 1 mM each of iodate (1), bromate (2), nitrite (3), bromide (4) and nitrate (5); PEG-monomolith M2 column (100×0.32 mm i.d.); 100 mM aqueous solution of various chloride eluent, as indicated; $4 \mu\text{L}/\text{min}$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

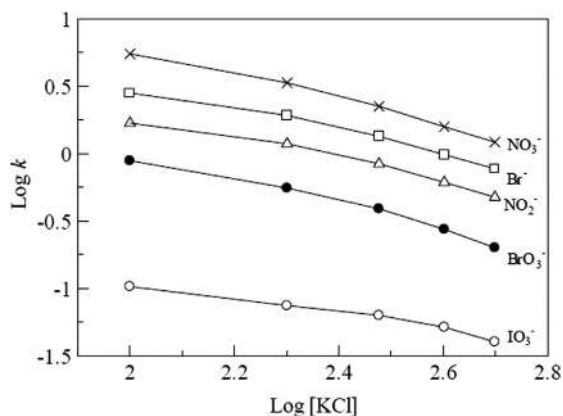


Fig. 5. Effect of potassium chloride concentration ($\log [\text{KCl}]$) on retention ($\log k$) of inorganic anions. Analytes, inorganic anions including 1 mM each of iodate (1), bromate (2), nitrite (3), bromide (4) and nitrate (5); PEG-monomolith M2 column (100×0.32 mm i.d.); 100 mM of potassium chloride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

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

Table 3

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

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

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

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

3.4. Mechanical stability

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

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

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

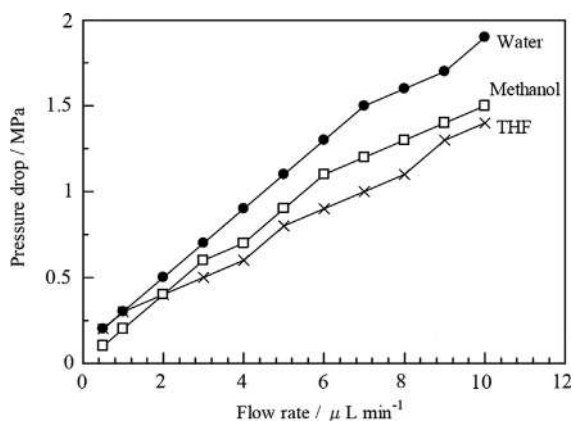


Fig. 6. Back pressure of M2 as a function of flow rate of water, methanol and THF.

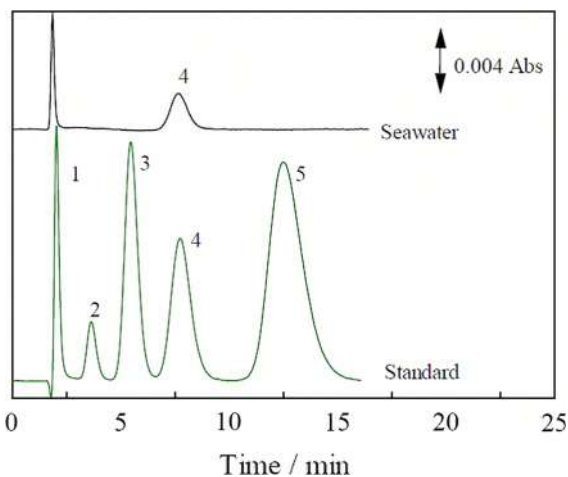


Fig. 7. Separation of anions contained in seawater sample. Operating conditions as in Fig. 3.

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

3.5. Application to real samples

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

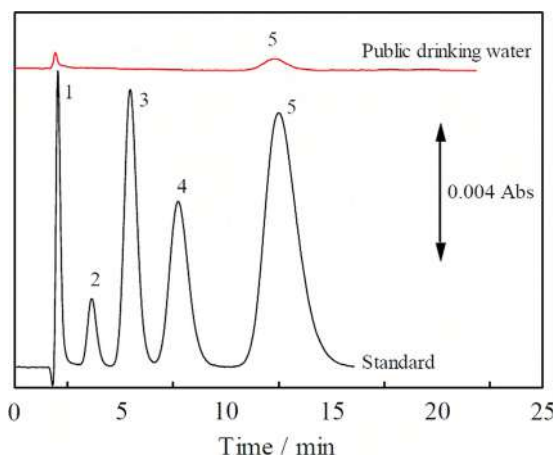


Fig. 8. Separation of anions contained in public drinking water sample. Operating conditions as in Fig. 3.

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

4. Conclusions

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

Appendix A. Supporting information

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

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Research Article

Preparation of a hybrid monolithic stationary phase with allylsulfonate for the rapid and simultaneous separation of cations in capillary ion chromatography

A hybrid monolithic column with sulfonate functionality was successfully prepared for the simultaneous separation of common inorganic cations in ion-exchange chromatographic mode through a simple and easy single-step preparation method. The strong cation-exchange moieties were provided directly from allylsulfonate, which worked as an organic monomer in the single-step reaction. Inorganic cations (Li^+ , Na^+ , K^+ , NH_4^+ , Cs^+ , Rb^+ , Mg^{2+} , Ca^{2+} , and Sr^{2+}) were separated satisfactorily by using CuSO_4 as the eluent with indirect UV detection. The allylsulfonate hybrid monolith showed a better performance in terms of speed and pressure drop than the capillary packed column. The number of theoretical plates achieved was 19 017 plates/m (in the case of NH_4^+ as the analyte). The relative standard deviations ($n = 6$) of both retention time and peak height were less than 1.96% for all the analyte cations. The allylsulfonate hybrid monolithic column was successfully applied for the rapid and simultaneous separation of inorganic cations in groundwater and the effluent of onsite domestic wastewater treatment system.

Keywords: Ion-exchange chromatography / Silica hybrid monolithic column / Strong cation exchangers / Sulfonate groups
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1 Introduction

Since monolithic columns appeared as a separation media in LC in the late 1980s, they have attracted great attention for wide application in capillary LC due to their advantages, such as easy preparation compared to conventional particle-packed columns and low backpressure [1–4]. Based on the precursors used, monolithic columns can be divided into two types: the organic polymer-based and inorganic silica-based columns. The former has good points such as easy preparation, good stability in a wide pH range and the flexibility of various combinations of monomers and cross-linkers. The latter provides good mechanical stability and good solvent resistance. However, its fabrication process is still time consuming.

Recently, organic–inorganic silica-hybrid monolithic columns have been developed rapidly as they combine the advantages of both silica- and organic polymer-based monolithic columns such as flexibility, low density, low cost, good stability, and long shelf life with excellent biocompatibility

and mechanical properties [5]. The incorporation of organic functional groups into the inorganic silica monolith matrix was useful for avoiding the time-consuming postmodification of common silica monoliths. A one-pot approach was introduced as a new strategy for more effective and sophisticated way in preparation of the monolithic column for achieving various target separations. After a report by Zou in 2009 [6], the one-pot approach has been developed in different temperature of precondensation as well as polymerization, then applied for the separation of enantiomers, proteins, peptides, and biomolecular species [7–10].

In LC, strong cation exchange (SCX) monolithic columns have been widely applied for the separation of proteins, peptides, and inorganic cations [11–13]. The SCX monolith was synthesized by in situ preparation of polymer-based organic and silica-based inorganic monolith columns with sulfonate functionalities arising from the versatile postmodifications and in situ chemical reactions. Generally, the detection of compounds were reported using a UV detector and a photodiode array detector. Nevertheless, hybrid monolith columns with SCX moieties have been very little reported in the literatures. Zou et al. prepared strong cation-exchange stationary phase for electrochromatography by a sol–gel process, which was then applied for the separation of β -blockers and alkaloids extracted from traditional Chinese medicines (TCMs) [14]. Zhang et al. successfully prepared a sulfonate hybrid monolithic column by a one-pot approach with a constant precondensation temperature but different polymerization temperatures; it is

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Abbreviations: AIBN, 2,2'-azobisisobutyronitrile; AS-hybrid, allylsulfonate hybrid; SAS, sodium allyl sulfonate; SCX, strong cation exchange; SP, swelling propensity; TMOS, tetramethoxysilane; THF, tetrahydrofuran; VTMS, vinyltrimethoxysilane

applied to proteome analysis [15]. Yang *et al.* reported that (potassium 3-sulfopropyl methacrylate)-silica hybrid monolithic columns have been prepared at the same temperature of precondensation and polymerization process. Then it was applied for the separation of different kinds of compounds including anilines, alkylbenzenes, and phenols by CEC [16]. Most of them were reported for applications of hybrid monolithic column in CEC. In this study, a hybrid monolithic column for the rapid and simultaneous separation of inorganic cations in ion-exchange mode will be the focus. The hybrid monolithic column with sodium allylsulfonate as the organic monomer will be prepared by a single-step thermal treatment one-pot manner. The polycondensation and polymerization will be carried out in short time at a single temperature for time-saving and high efficiency purposes. Sulfonate groups as SCX moieties were provided from sodium allylsulfonate and they could be directly added to the hydrolysis solution. Then the optimized condition of allylsulfonate (AS)-hybrid monolithic column was applied for simultaneous determination of cations in groundwater as well as effluent of domestic wastewater treatment system in capillary ion chromatography. To the best of our knowledge, this could be the first time that strong cation-exchange hybrid monolithic columns have been established for rapid and simultaneous determination of inorganic cations in IEC mode.

2 Materials and methods

2.1 Reagents and materials

Tetramethoxysilane (TMOS), vinyltrimethoxysilane (VTMS), sodium allylsulfonate (SAS), 2,2'-azobisisobutyronitrile (AIBN), and urea were obtained from TCI (Tokyo, Japan). PEG (M.W.10000) was obtained from Aldrich (Rockford, IL, USA). Copper(II) sulfate was of extra pure reagent grade (Nacalai Tesque, Kyoto, Japan). Purified water was produced in the laboratory by using a GS-590 water distillation system (Advantec, Tokyo, Japan). All other reagents used in the experiment were of guaranteed reagent grade. All the cation solutions used in this study were prepared from extra pure reagents obtained from Nacalai Tesque.

2.2 Preparation of AS-hybrid monolith

The fused-silica capillary tube (0.320 mm id \times 0.450 mm od) was purchased from GL Sciences (Tokyo, Japan). Before use, the fused-silica capillary tube was washed sequentially with 1 M NaOH solution, deionized water, 1 M HCl and deionized water for 30 min each, and dried by nitrogen at room temperature for further use. The AS-hybrid monolithic column was prepared by mixing and stirring TMOS (0.18 mL), VTMS (0.06 mL), acetic acid (0.5 mL), urea (45 mg), and PEG (10 000 MW, 54 mg) at 0°C for 1 h and hydrolyzing it to form a homogenous solution. Then 35–50 mg of SAS and 2 mg of AIBN were added into the mixture solution with

15 min of sonication for mixing. After that, it was manually introduced into the pretreated capillary tube to the appropriate length by syringe and both ends of the tube were sealed. The condensation and polymerization were carried out at 60°C for 12 h. The obtained SCX hybrid monolith columns were then flushed with methanol to remove the PEG and other residuals.

2.3 Apparatus

The chromatography separation was carried out using a capillary LC system constructed by an L.TEX-8301 Micro Feeder (L.TEX, 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 μ L (Rheodyne, Cotati, CA, USA), a 100 mm \times 0.32 mm id of microcolumn and a UV-2075 intelligent UV/VIS detector, (JASCO, Tokyo, Japan) that was operated at 210 nm. The data were acquired using a Chromatopac C-R7A data processor (Shimadzu, Kyoto, Japan). The inlet pressure was monitored with L.TEX-8150 Pressure Sensor (L. TEX). The morphology of the column was characterized by an S-4800 scanning electron microscope (Hitachi, Tokyo, Japan). The characteristics of surface area and pore size distribution were measured by 3-Flex Surface Characterization Analyzer (Micrometrics, USA).

3 Results and discussion

3.1 Preparation of AS-hybrid monolithic column

In this research, the AS-hybrid monolithic column was prepared based on one-pot manner of simultaneous mixing of silane monomers and organic monomers. The scheme of the expected reaction for preparing the sulfonate hybrid is based on two major reactions, as shown in Fig. 1: the polycondensation of hydrolyzed precursors of TMOS and VTMS, and the copolymerization of SAS and vinyl organic monomers. The temperature is one of the sensitive factors in the preparation of AS-hybrid monolith during polycondensation that can give a good performance and permeability. The columns were synthesized at 50°C (column F) to 80°C (column H) with AIBN as the initiator. The mechanical stability results of the AS-hybrid monolith at different temperatures of polycondensation were shown in Table 1. By changing the temperature from 50 to 80°C at the same reaction time 12 h, the permeability value decreased from 11×10^{-12} to 5.5×10^{-12} m² and the pressure drop as well increased from 0.2 to 0.4 MPa (under operational condition 2 μ L/min). The preparation of AS-hybrid monolith at 60°C was found the highest value of theoretical plates 19 017 plate/m (in the case of NH₄⁺ as the analyte). Based on this, the temperature at 60°C was then applied in the following preparation of AS-hybrid monolith.

The organic monomer and silane were simultaneously mixed in the developed “one-pot” manner. The quantity of

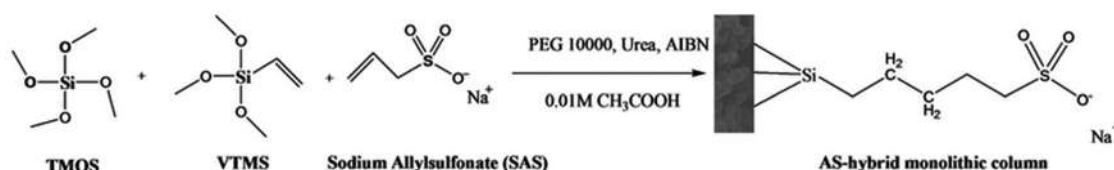


Figure 1. Schematic of expected reaction for the preparation of AS-hybrid monolithic column.

Table 1. Effects of preparation parameters on the fabrication of AS-hybrid monolith column

Column	SAS ^{a)} (mg)	Time (h)	Temp (°C)	Back Pressure ^{b)} (MPa)	<i>k</i> Permeability 10 ⁻¹² (m ²)	<i>N</i> _{max} (plates/m)
A	35	9	60	0.2	11	6107
B	35	12	60	0.2	11	19 017
C	35	18	60	0.5	4.4	4214
D	35	24	60	0.6	3.3	12 707
E	35	36	60	0.8	2.6	8603
F	35	12	50	0.2	11	15 869
G	35	12	70	0.3	7.3	19 001
H	35	12	80	0.4	5.5	15 636
I	30	12	60	0.2	11	8795
J	40	12	60	0.2	11	14 100
K	45	12	60	0.2	11	14 220
L	50	12	60	0.2	11	14 618

a) Sodium allylsulfonate (SAS) as organic monomer.

b) System operated with CuSO₄ with flow rate 2 μL/min.

organic monomer in this reaction should be examined because it affects the formation of the hybrid monolith column. The precondensation mixture was prepared with different amounts of SAS, i.e. 30, 35, 40, 45, and 50 mg. As shown in Table 1, the permeability values of the column were stable at $11 \times 10^{-12} \text{ m}^2$ when the amount of SAS were 30, 35, 40, 45, and 50 mg (column I, B, J, K, and L). Additionally, the theoretical plates were different by changing the amount of SAS. By these results, the optimum amount of SAS that provided the best theoretical plates in precondensation mixture

was 35 mg (in the case of NH₄⁺ as the analyte). Further increased amount of SAS would result increasing the amount of unreacted residuals that are difficult to be eluted during the preparations of AS-hybrid monolith.

Figure 2 shows the relationship of preparation time and the amount of sulfonate groups (denoted as cation-exchange capacity) of the AS-hybrid monolith. The precondensation and prepolymerization conditions were kept at 60°C and 35 mg as the amount of SAS was added. As seen in Fig. 2, the cation-exchange capacities of the AS-hybrid monolith columns (A–E) that were prepared for 9, 12, 18, 24, and 36 h were 0.74, 0.80, 0.81, 0.82, and 0.83 mequiv/mL, respectively. The capacity linearly increased by the longer preparation time of up to 10 h and was nearly constant at longer preparation time. These indicated that the capability of a monolithic column as AS-hybrid monolith could be successfully achieved by changing preparation time of the AS-hybrid monolith, and these monolithic capillary columns could be produced by different capacities of up to 0.83 mequiv/mL.

3.2 Separation of common inorganic cations on ion-exchange mode

The sulfonate groups of the AS-hybrid monolithic column work as a strong cation-exchange site that interacts with positively charged compounds through electrostatic interaction. The common inorganic cations were used to examine the cation-exchange characteristic of the AS-hybrid monolith. The common cations were indirectly detected with UV absorption at 210 nm and copper(II) sulfate as eluent. As

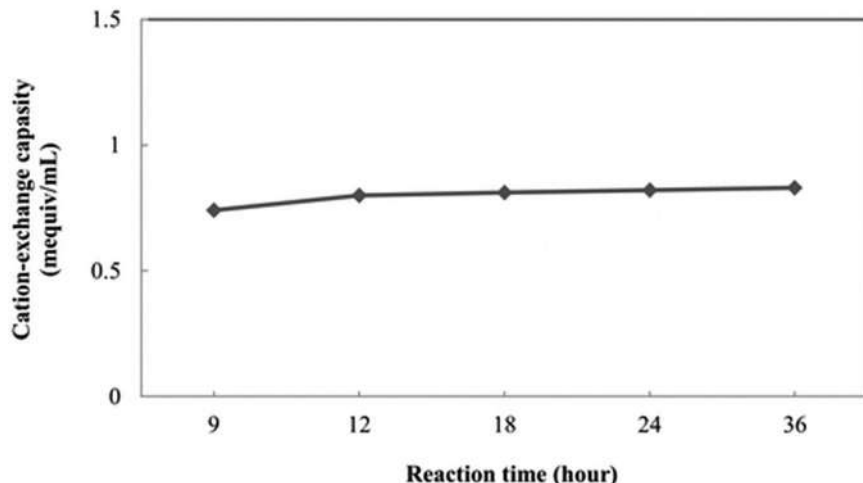


Figure 2. The relationship of preparation time and the amount of sulfonate groups (denoted as cation-exchange capacity) of the AS-hybrid monolith.

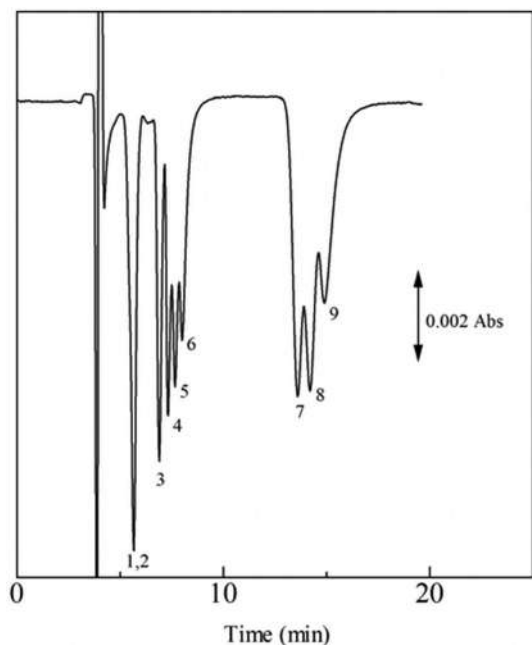


Figure 3. Separation of inorganic cations on the AS-hybrid monolith. Operating conditions: Analytes, 1 mM each of Li⁺ (1), Na⁺ (2), NH₄⁺ (3), K⁺ (4), Rb⁺ (5), Cs⁺ (6), Mg²⁺ (7), Ca²⁺ (8), and Sr²⁺ (9); AS-hybrid monolith column (100 × 0.32 mm id); 5 mM of CuSO₄ eluent; 2 μL/min flow rate; 210 nm wavelength of UV detection and 0.2 μL injection volume.

shown in Fig. 3, common inorganic cations such as Li⁺, Na⁺, NH₄⁺, K⁺, Rb⁺, Cs⁺, Mg²⁺, Ca²⁺, and Sr²⁺ were separated well except peaks of overlapped Li⁺ and Na⁺. These suggest that the cation-exchange capacity of the AS-hybrid was not high enough to separate the Li⁺ and Na⁺ because the ionic radius of Li⁺ (0.090 nm) is close to Na⁺ (0.116 nm). The resolution between two cations, i.e. Mg²⁺, Ca²⁺ of the AS-hybrid was 0.66. Furthermore, the separation performance of cations was better for some peaks when longer capillary columns were used (15 cm of length), and the resolution between two cations, i.e. Mg²⁺, Ca²⁺ was 0.96. However, changing the length of capillary column did not show the significant changes for the separation of Li⁺ and Na⁺. The separation was carried out at flow rate 2 μL/min with 0.2 MPa of pressure drop. In addition, the effect of copper(II) sulfate concentration (log [CuSO₄]) as the eluent on the retention factor (log *k*) was investigated. Figure 4 shows the logarithm of the retention factor (log *k*) of cations as the function of the logarithm of the eluent concentrations. The retention of each cation decreased with increasing the concentration of eluent, which indicates that the separation mechanism of the cations on the AS-hybrid monolith involved cation exchange.

3.3 Comparison of AS-hybrid and particle-packed capillary

To know the performance of AS-hybrid column, the mechanical stability of an AS-hybrid monolithic capillary column was

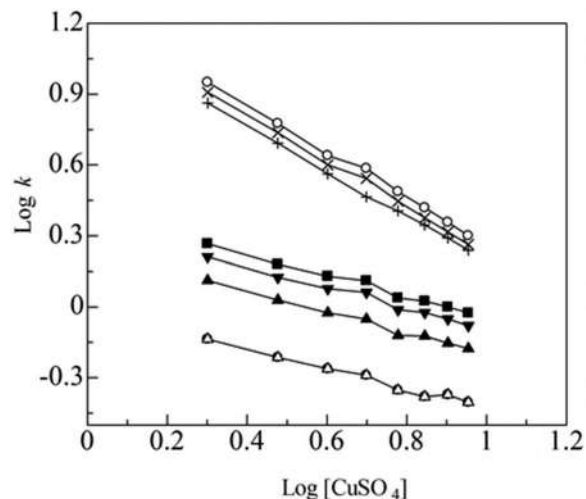


Figure 4. Effect of copper(II) sulfate concentration (log [CuSO₄]) on retention (log *k*) of inorganic cations. The symbols in the figure represent as (●) Li⁺, (Δ) Na⁺, (▲) NH₄⁺, (□) K⁺, (▼) Rb⁺, (■) Cs⁺, (+) Mg²⁺, (×) Ca²⁺, (○) Sr²⁺. Operating conditions as in Fig. 3.

compared with a particle-packed capillary column. As a packing material, IC cation SW (5 μm; Tosoh, Tokyo, Japan) was packed on a 100 mm × 0.32 mm id microcolumn. Figure 5 shows the separation performance of IC cation SW under operating conditions for 5 mM CuSO₄ as the eluent and 2 μL/min as the flow rate. The packing material could separate the common inorganic cations especially for monovalent (Li⁺ and Na⁺) as well as divalent (Mg²⁺ and Ca²⁺) whereas NH₄⁺, K⁺, Rb⁺, and Cs⁺ were poorly separated. The higher cation-exchange capacity of the particle-packed column than AS-hybrid might be one of the reasons why the separation was better. The packed capillary column with cation-exchange capacity around 0.99 mequiv/mL had lower

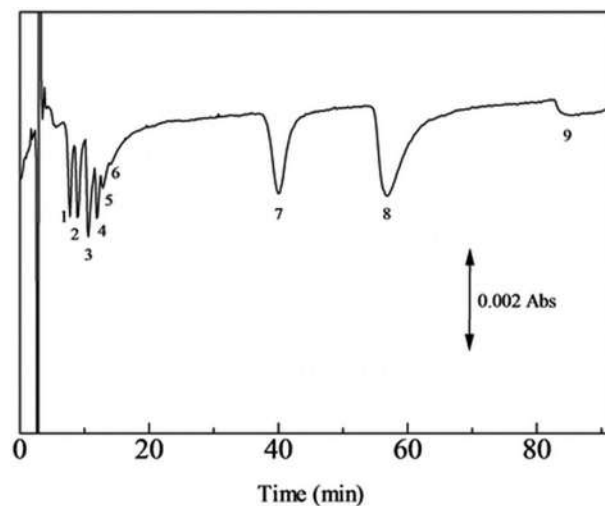


Figure 5. Separation of inorganic cations on a cation-exchange packed capillary column. Packing material, IC cation SW (5 μm) packed into 100 × 0.32 mm id. Operating conditions as in Fig. 3.

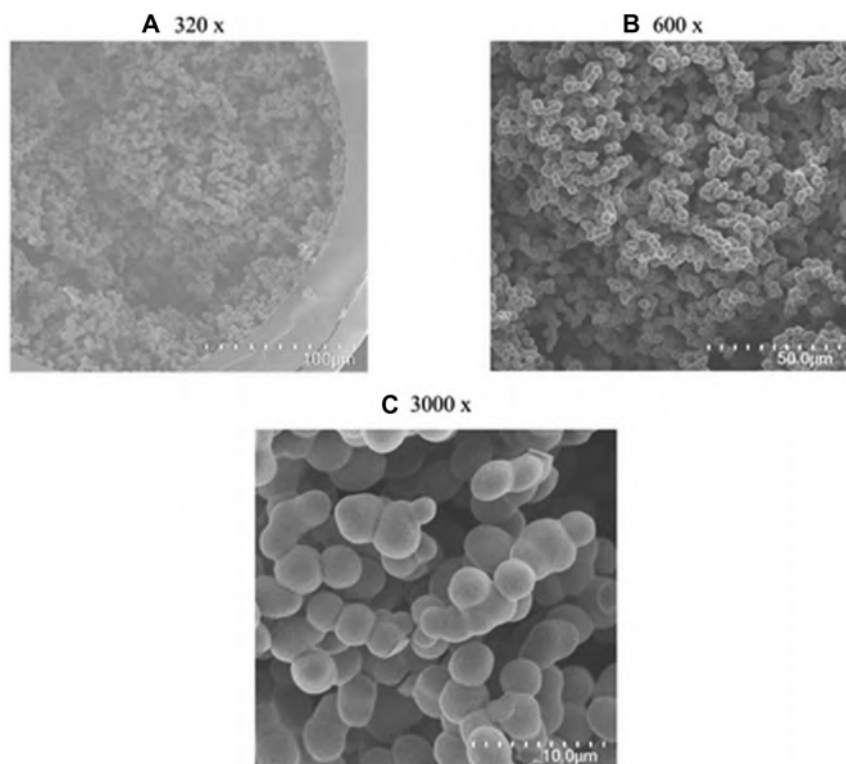


Figure 6. SEM images of AS-hybrid monolith magnification = 320× (A), 600× (B), and 3000× (C).

theoretical plate (11 240 plates/m) compared to the AS-hybrid (19 017 plates/m; in the case of NH₄⁺ as the analyte). In addition, the pressure drop for the packed capillary column reaches 1.5 MPa and the elution time was more than 90 min. On the contrary, AS-hybrid only reached 0.2 MPa and the elution time was only 15 min. These results indicate that the AS-hybrid monolith has better performance in terms of speed and the pressure drop than the packed capillary column under the same operating conditions.

3.4 Characterization and mechanical stability of the AS-hybrid monolithic column

After optimization of the preparation condition, the AS-hybrid monolith was subsequently characterized. The SEM photos of AS-hybrid monolith capillary column prepared

under the optimum condition are shown in Fig. 6. It is shown that the AS-hybrid monolith formed is attached to the inner wall of the capillary tube. It is because the silanol groups at the inner wall of the capillary tube are an important part in the polycondensation during preparation of AS-hybrid monolith column [17]. A uniform organic-silica hybrid monolithic matrix with large through-pores was obtained, leading to little pressure drop as shown in Fig. 6B and 6C. The effect of the flow rate on the pressure drop across the AS-hybrid monolith column for four different solvents was displayed in Fig. 7. This figure shows that the pressure drop of each solvent has good linear relationship with flow rate. When the flow rate was raised up to 10 μL/min, the pressure drop increased maximum up to 2.6 MPa (for THF, tetrahydrofuran), this means that the AS-hybrid monolith had good performance and could be operated at higher flow rate and less pressure drop.

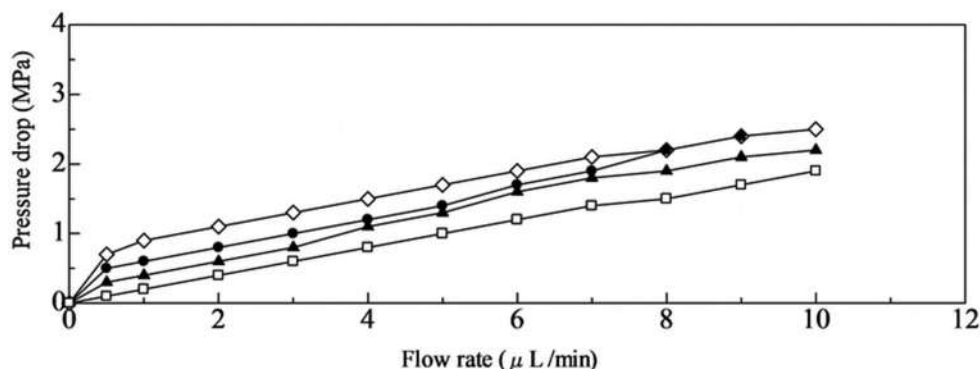


Figure 7. Backpressure of column B as a function of flow rate of (◇) water, (▲) methanol, (□) acetonitrile, and (●) THF.

Table 2. Summaries data for the retention time (t_R), RSDs, and LODs of common inorganic cations

Cations	t_R (min)	RSD (% , $n = 6$)		LOD
		Retention time	Peak height	S/N = 3 (ppm)
Li ⁺	5.70	0.49	1.96	0.09
Na ⁺	5.70	0.49	1.96	0.09
NH ₄ ⁺	7.00	0.59	1.83	0.08
K ⁺	7.52	0.57	1.90	0.21
Rb ⁺	7.88	0.53	1.94	0.50
Cs ⁺	8.21	0.40	1.71	0.93
Mg ²⁺	13.70	0.51	1.96	0.14
Ca ²⁺	14.40	0.60	1.85	0.23
Str ²⁺	15.30	0.52	1.95	0.72

Operation condition as in Fig. 3.

The mechanical stability of the AS-hybrid monolith was examined by calculating the permeability and the swelling propensity (SP) factor. By plotting the pressure drop against the flow rate, permeability and SP factor could be obtained. The calculation of the permeability (B_0) followed Darcy's law Eq. (1) [18], where F is the flow rate of the mobile phase, η is the viscosity of the mobile phase, L is the effective length of the column, r is the inner radius of the column and ΔP is the pressure drop across the column. The permeability was measured by pumping water through the column with increasing the flow rate from 0.5 to 4 $\mu\text{L}/\text{min}$, leading to the increase in

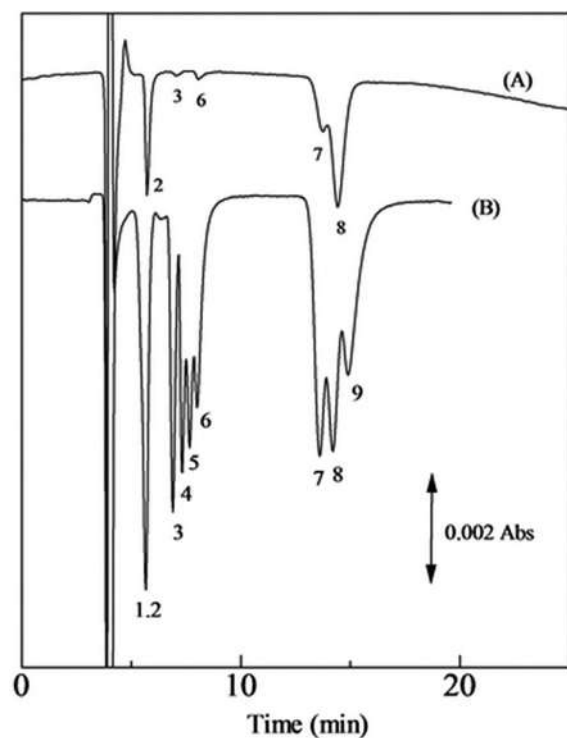


Figure 8. Separation of cations contained in groundwater sample. Groundwater sample (A); standard inorganic cations (B); operating conditions as in Fig. 3.

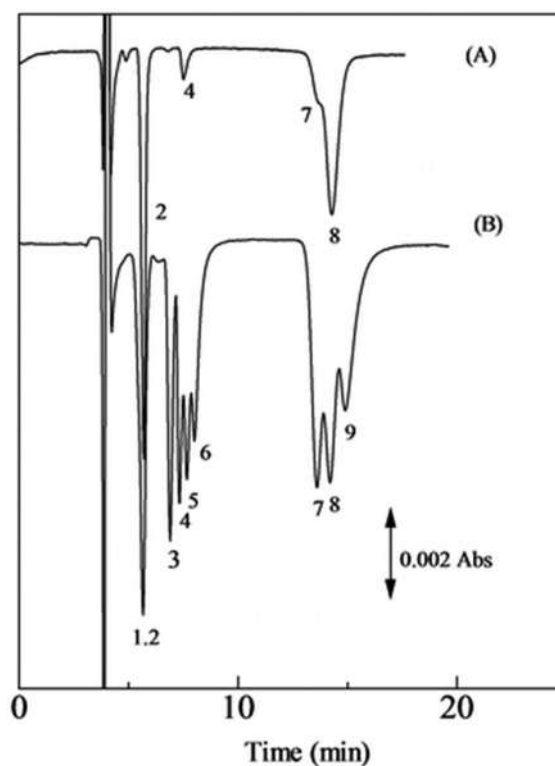


Figure 9. Separation of cations contained in the onsite domestic wastewater treatment system sample. The onsite domestic wastewater treatment system sample (A); standard inorganic cations (B); operating conditions as in Fig. 3.

the pressure drop from 0.1 to 0.4 MPa. The permeability value of AS-hybrid monolith was $11 \times 10^{-12} \text{ m}^2$ under optimum operation condition (flow rate; 2 $\mu\text{L}/\text{min}$). This indicated that the AS-hybrid monolith can run excellently with mechanical stability under high pressure drop.

$$B_0 = \frac{F\eta L}{\pi r^2 \Delta P} \quad (1)$$

$$SP = \frac{p(\text{solvent}) - p(\text{water})}{p(\text{water})} \quad (2)$$

The pore structure of the AS-hybrid monolithic column stationary phase was characterized in terms of pore size distribution and surface area that was determined by nitrogen adsorption isotherms method. A narrow size distribution was observed at approximately 1 nm with the pore volume of 0.5 cm^3/g and the BET surface area was measured to be ca. 164 m^2/g . The pore size in the AS-hybrid monolithic column was smaller than the other hybrid columns [19, 20], which may be associated to the presence of the sulfonate groups on the surface of pore in the hybrid monolithic column.

The swelling propensity (SP) was calculated by using the Eq. (2) [21], where p is the pressure drop relative to the viscosity, $p = \Delta P/\eta$. In general, the SP value of nonswelling material

is zero. The swelling occurs more when the SP shows higher value whereas a negative value of SP indicates shrinkage of the monolith [22]. In this study, the SP value of AS-hybrid monolith (Column B) was determined by using water as the eluent for 30 min and the pressure drop was marked. The eluent was then changed to THF and waits until the system became stable, and the pressure drop was marked. Acetonitrile was also used the same way as water in determination of SP. The SP value determined by applying THF and acetonitrile was found to be around 0.64, and 0.11, respectively. These values indicated that AS-hybrid monolith shows no significant swelling and shrinkage of the AS-hybrid monolith rod observed, hence these monoliths could work on different polarities with the high flow rate compared to the packing materials reported with SP values from 0.05~7.4 [19].

3.5 Reproducibility of AS-hybrid monolithic column

The repeatability of the retention time (t_R) and the peak height were calculated under the optimum conditions as seen in Table 2. The RSDs, for $n = 6$ of the retention time and peak height were found less than 1.96% for all the analyte cations. This value showed the satisfactory performances reproducibility of the AS-hybrid monolith. Based on this result, the AS-hybrid monolith in our study showed better improvement on performance than in other previous literatures that determination of common inorganic cations using diol-group bonded silica gel with 1,3-propanesultone [23], which indicated that the AS-hybrid monolith columns had exceptional reproducibility, and the preparation of organic-inorganic hybrid monolithic column using “one-pot” approach manner was feasible.

In addition, the limits of detection of these nine common inorganic cations were determined by injection of 0.2 μ L volume of the sample, 1 mM each of cation, and the detection limits were calculated at $S/N = 3$. The detection limits obtained by this method ranged from 0.09–0.93 ppm for the cations as shown in Table 2.

3.6 Application to real samples

The one-pot approach method of AS-hybrid monolith columns were applied for the determination of common inorganic cations in the real samples. Two different samples, one is groundwater sample from Padang City, Indonesia, and another is the effluent of onsite domestic wastewater treatment system that was collected from Gifu city, Japan, were used for application. The water samples were examined by the method within a few days after collection. As a pretreatment, the samples were filtered through a 0.45 μ m membrane filter for ion chromatography samples. Figure 8 shows chromatograms of groundwater and standard mixture of nine common inorganic cations. It can be seen that these inorganic cations are properly detected. The concentrations of inorganic cations for

Na^+ , NH_4^+ , Cs^+ , Mg^{2+} , and Ca^{2+} were 9.1, 1.6, 5.7, 6.3, and 41.9 ppm, respectively. On the other hand, a chromatogram of the effluent of onsite domestic wastewater treatment system for Na^+ , K^+ , Mg^{2+} , and Ca^{2+} was successfully separated, where the concentration of each cation was 27.5, 4.5, 3.5, 28.3 ppm, respectively (Fig. 9).

4 Concluding remarks

An AS-hybrid monolithic capillary column with strong cation-exchange moieties was successfully fabricated for the simultaneous separation of common inorganic cations in ion-exchange chromatographic mode. The simple and easy preparation with incorporation of sodium allylsulfonate into the silica monolith matrix was carried out by single-step thermal-treatment one-pot manner. Nine common inorganic cations (Li^+ , Na^+ , NH_4^+ , K^+ , Rb^+ , Cs^+ , Mg^{2+} , Ca^{2+} , and Sr^{2+}) could be satisfactorily separated with sufficient RSDs and low limits of detection. The satisfactory performances of AS-hybrid monolith columns in mechanical stability were proved by the fact that no significant swelling or shrinkage occurred in the columns and it could also work in solutions with different polarities at high flow rates. This method showed the elution of cations within 15 min with the pressure drop of only ca. 0.2 MPa, which has better performance in terms of speed and the pressure drop than the particle-packed capillary column. Based on the results in our work and compared to other literatures, we found a novel application of an AS-hybrid monolithic column for rapid and simultaneous determination of both common monovalent and divalent inorganic cations in ion-exchange chromatography mode.

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5 References

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