

Separation of Inorganic Anions Using Methacrylate-Based Monolithic Column Modified with Trimethylamine in Ion Chromatography Capillary System

Fitri Mairizki¹, Athika Rahmah¹, Hilma¹, Radhia Putri¹, Rahmiana Zein^{1*},
Lee Wah Lim², Toyohide Takeuchi^{2*}, Edison Munaf¹

¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, Indonesia

²Department of Chemistry, Faculty of Engineering, Gifu University, Gifu, Japan

Email: *rzejn@fmipa.unand.ac.id

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ABSTRACT

Methacrylate-based monolithic column was prepared in fused-silica capillary (80 × 0.32 mm i.d.) by *in situ* polymerization reaction using glycidyl methacrylate as monomer; ethylene dimethacrylate as crosslinker; 1-propanol, 1,4-butanediol, and water as porogenic solvents. The monolith matrix was modified with trimethylamine to create strong anion exchanger via ring opening reaction of epoxy groups. The morphology of the monolithic column was studied by using Scanning Electron Microscope (SEM). This column had good mechanical stability and permeability. The effects of various mobile phases for separation of inorganic anions were investigated. Iodate, bromate, nitrite, bromide, and nitrate were separated within 11 min using 100 mM potassium chloride as mobile phase and detected at 210 nm. This method showed good precision of retention time, acceptable linearity and good sensitivity. Under the optimum condition, the RSD of the retention time was in the range of 1.09% - 1.75% ($n = 6$). The calibration curve showed linear relationships between the peak area and the concentration. The limits of detection (LOD) and the limits of quantitation (LOQ) were between 0.08 - 0.18 mM and 0.26 - 0.61 mM, respectively. This method was applied to the determination of inorganic anions in tap water and ground water samples.

Keywords: Methacrylate-Based Monolithic Column; Trimethylamine; Ion Chromatography Capillary System; Inorganic Anions; Water Sample

1. Introduction

Ion chromatography was first introduced by Small, *et al.* in 1975 as a new analytical method. In a short time, ion chromatography has grown rapidly as one of the technologies for the separation and determination of inorganic anions and cations, carbohydrates, small organic compounds, peptides or proteins. In ion chromatography, the stationary phases have a functional group that will interact with the ionic analytes through electrostatic interactions so as the ionic analytes can be separated [1]. Zein, *et al.* and Munaf, *et al.* [2,3] developed bovine serum albumin as a stationary phase for the separation of inorganic anions. A few papers also have been reported for the simultaneous separation of anions and cations [4-6].

Various types of stationary phase have been developed

for the separation of ionic species. In recent years, monolithic columns have attracted much attention as the separation media in chromatography. Monolithic columns in capillary liquid chromatography were introduced by Hjerten, *et al.* in 1989, and since that time, monolithic columns have rapidly become highly popular [7]. Monolithic columns have many unique properties. They have porous structure with bimodal pore size distribution, nm-sized mesopores at the surface of the skeletal structure and μm -sized through pores (macropores) between the skeletal structure. The nm-sized mesopores can give sufficient surface area, which have a positive effect on separation efficiency. On the other hand, the μm -sized through pores generate low-pressure drop and enhance mass transfer kinetics which can allow the use of higher flow rates for rapid separations on relatively long columns [8]. The diffusion path length and flow resistance can be reduced using monolithic columns compared with

*Corresponding author.

a particle-packed. The ease of preparation and availability of various precursors also contributes to the popularity of monoliths [9].

A greater chromatography efficiency is shown by the monolithic columns for the separation of macromolecules such as nucleotides, oligonucleotides [10], peptides [11] and proteins [12-15]. Therefore, the monolithic columns have been applied in environmental, pharmaceutical, and genomic applications [16]. Recently, a few researchers began to explore the monolithic columns application for the separation of small organic molecules [17-19], and inorganic molecules such as ionic species [20,21].

In general, there are two types of monolithic columns which have been developed for chromatography: silica-based monolithic column [22-24] and organic polymer-based monolithic column [25,26]. Silica-based monolithic columns are produced by using the sol-gel approach to make a continuous sol-gel network throughout the column. On the other hand, organic polymer-based monolithic columns are produced by *in situ* polymerization. The polymerization reaction mixtures consist of a combination of an initiator, monomers, cross-linkers and porogens [27]. The organic polymer-based monolithic columns have some advantages compared with silica-based monolithic columns, including simple preparation process, high stability over a wide pH range and easily modified for different purposes [28,29]. However, the organic polymer-based monolithic column applications for separation of ionic species had still remained a challenge in ion chromatography.

There are three types of organic polymer-based monolithic columns: acrylamide-based, methacrylate-based, and styrene-based polymers [30]. The methacrylate-based polymers have some advantages related with their use as stationary phase in monolithic columns, such as simple preparation, easy functionalization, various selectivity, and high stability under extreme pH conditions (pH 2 or 12) [31,32]. The combination of their properties makes methacrylate-based monolithic columns become new interesting opportunities in chromatography for the analysis of complex matrices and fast separation [33].

In this work, we use glycidyl methacrylate as monomer to prepare methacrylate-based monolithic column, because it has highly reactive epoxy ring which could be easily converted into anion exchange group via ring opening reaction. The present paper describes the use of methacrylate-based monolithic column which modified with trimethylamine to produce a strong anion exchanger for separation of inorganic anions.

2. Experimental

2.1. Apparatus

Chromatographic evaluation was performed using a microfeeder (L. TEX Corporation, Tokyo, Japan) equipped

with a gas-tight syringe (0.5 mL; Ito, Fuji, Japan) as a pump, a micro valve injector with an injection volume 0.2 μL (Upchurch Scientific, Oak Harbor, WA, USA), a 80×0.32 mm i.d. as a separation column (GL Science, Tokyo, Japan), a UV detector (Jasco, Japan) with a modified flow cell and Chromatopac C-R7Ae as a recorder (Shimadzu, Kyoto, Japan). The flow rate of the mobile phase was kept constant at 4 $\mu\text{L}\cdot\text{min}^{-1}$. Scanning electron micrographic images for morphology observation of the monolithic column were obtained using a SEM S-4800 (Hitachi, Japan).

2.2. Chemicals

All chemicals used were of analytical grade. 2,2'-azo-bis(isobutyronitrile) (AIBN), glycidyl methacrylate (GMA) and ethylene dimethacrylate (EDMA) were purchased from Wako Pure Chemical Industries (Osaka, Japan). 1-propanol and 1,4-butanediol were obtained from Nacalai Tesque (Kyoto, Japan). Trimethylamine (30 wt% in H_2O) and tetrahydrofuran were from Kanto Chemical (Tokyo, Japan). The purified water was prepared using GS-590 water distillation system (Advantec, Tokyo, Japan). Other chemicals were used as received.

2.3. Preparation of Methacrylate-Based Anion-Exchange Monolithic Column

The fused silica capillary was first activated by 1 $\text{mol}\cdot\text{L}^{-1}$ NaOH, purified water, and 1 $\text{mol}\cdot\text{L}^{-1}$ HCl. 3-(trimethoxysilyl)-propyl methacrylate (γ -MAPS) solution (30% (v/v) in acetone) was used to fill the activated capillary. After sealing the capillary two ends, the reaction was allowed to perform 60°C for 24 h in waterbath. Then, the capillary was washed thoroughly with acetone. The N_2 was flown through the capillary to dry the inner surface before further use.

The monolithic column was prepared by *in situ* polymerization. A polymerization mixture containing GMA (30% (v/v)), EDMA (10% (v/v)), 1-propanol (35% (v/v)), 1,4-butanediol (20% (v/v)), water (5% (v/v)), and AIBN (1% (w/v) of the total monomer amount). This mixture solution was ultrasonically homogenized for 5 min and immediately aspirated into the pretreated capillary. After sealing both ends, the treated capillary was placed in the waterbath to proceed the polymerization at 60°C for 24 h. The monolith was washed with methanol to remove the unreacted monomers and remaining porogenic solvent present in the column. Subsequently, epoxy groups in the monolith were reacted with trimethylamine to get strong anion exchanger, as the following procedure: a trimethylamine solution (50% (v/v) in tetrahydrofuran) was passed through the monolithic column at flow rate 4 $\mu\text{L}\cdot\text{min}^{-1}$ for 2 h. Then, the monolithic column was placed in the oven to proceed the modification at 80°C

for 5 h. The anion exchange monolithic column produced was washed with methanol for 3 h at $2\mu\text{L}\cdot\text{min}^{-1}$ of flow rate.

3. Results and Discussion

3.1. Preparation of Methacrylate-Based Anion-Exchange Monolithic Column

The monolithic column was prepared directly in the capillary by *in situ* polymerization method. There are two steps were adopted in this experiment to form the polymer network. First, the synthesis of a rigid polymer matrix by using GMA as monomer, EDMA as crosslinker, and ternary porogen which consists of 1-propanol, 1,4-butanediol, and water. Then, the introduction of trimethylamine as strong anion exchange moiety via ring-opening reaction of the epoxy group. The composition of the monomer, crosslinker, porogen solvent; polymerization reaction time; and modification conditions would have great effect on the monolithic structure.

The morphology of the monolithic column was an important parameter that can affect the capability and efficiency separation. The morphology of the monolithic column was examined by scanning electron microscopy (SEM). **Figure 1(a)** demonstrates that the morphology of monolithic column were solid and completely attached to

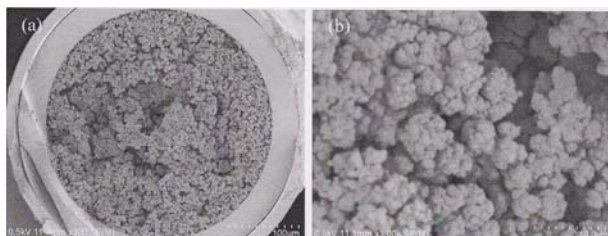


Figure 1. Scanning electron microphotographs of monolithic column. (a) Wide-view and (b) close-up-view.

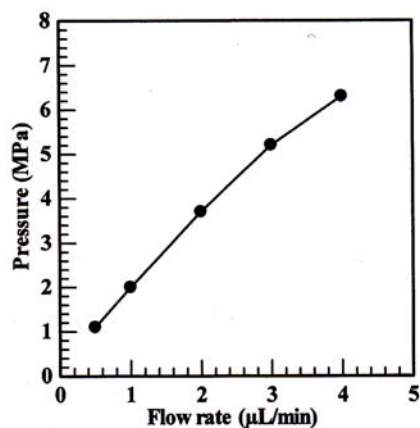


Figure 2. Plots of the flow rate of water against the back pressure of the monolithic column. Column, 80×0.32 mm i.d.; mobile phase: water.

the inner surface of the capillary column. It indicated that the monolith was covalently bonded to the capillary. The successful attachment of monolith into the inner surface of the capillary column influenced by the pre-treatment step of the capillary column. As shown in **Figure 2(b)**, the obtained monolith displayed porous network with globular structure. The continuous porous channels in the monolith bed which were formed by mesopores and through pores can also be seen.

The permeability of monolithic column was examined by measure the back pressure for different flow rate using water as mobile phase. The flow rate ranged from 0.5 to $4\mu\text{L}\cdot\text{min}^{-1}$. Column permeability is affected by amount of porogenic solvent. A sufficient amount of porogen would result good permeability so as the mobile phase and sample solution would flow through the column under small back pressure. On the contrary, insufficient amount of porogen would make the mobile phase and sample solution flow through the column under large back pressure [34]. The permeability of the monolithic column was calculated as $9.88 \times 10^{-13}\cdot\text{m}^2$ which indicated that the monolithic column had good permeability. The permeability (B_0) was calculated by using Darcy's Law [35],

$$B_0 = F\eta L / (\pi r^2 \Delta P)$$

where F was the linear velocity of the mobile phase, η was the dynamic viscosity of the mobile phase ($\eta = 0.089$ Pa s for water), L was the effective column length, and ΔP was pressure drop.

On the other hand, the back pressures dependence on flow rate was a straight line with a correlation coefficient R^2 0.992 (**Figure 2**), which indicated the good mechanical stability of the prepared monolithic column.

3.2. Separation of Inorganic Anions

Three anions were first used to evaluate the performances of the monolithic column. **Figure 3** demonstrates the separation of three inorganic anions on monolithic column using various mobile phases with the same concentration. The analytes are detected at 210 nm. All the analytes can well separated. Potassium chloride provided better resolution of the anions in a shorter retention time and more reproducible signals than the others.

In order to increase the retention time of analytes, the concentration of the mobile phase should be higher. Potassium chloride was examined as the mobile phase in the 50 - 200 mM concentration range. Separations of five inorganic anions were shown in **Figure 4**. The elution order was iodate, bromate, nitrite, bromide and nitrate. The present system is more sensitive to determination of iodate, nitrite and nitrate compared with bromate and bromide. It's seen that, the retention time of analytes could be increase with the increasing of the mobile phase

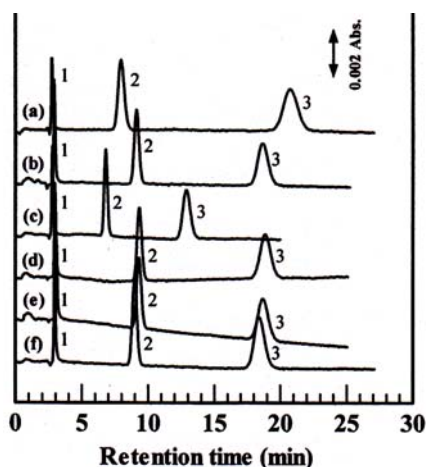


Figure 3. Effect of the mobile phase on the separation of inorganic anions. Column, 80×0.32 mm i.d.; mobile phase: 50 mM LiCl 50 (a), 50 mM NaCl (b), 50 mM KCl (c), 50 mM RbCl (d), 50 mM CsCl (e), 50 mM NH_4Cl (f); flow rate: $4 \mu\text{L}\cdot\text{min}^{-1}$; injection volume, 0.2 μL ; wavelength of UV detection: 210 nm; analytes: 1 = iodate, 2 = nitrite, 3 = nitrate, 1.0 mM each.

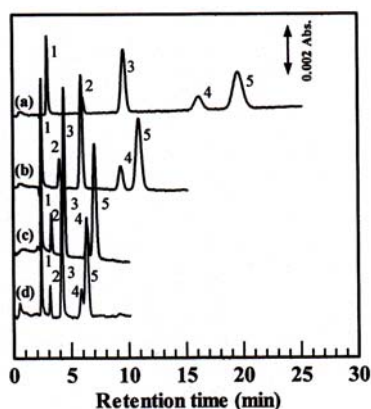


Figure 4. Effect of concentration of potassium chloride mobile phase on the separation of inorganic anions. Column dimension: 80×0.32 mm i.d.; concentration of mobile phase: (a). 50 mM, (b). 100 mM KCl, (c). 150 mM, (d). 200 mM; flow rate: $4 \mu\text{L}\cdot\text{min}^{-1}$; injection volume: 0.2 μL ; wavelength of UV detection, 210 nm; analytes, 1 = iodate, 2 = bromate, 3 = nitrite, 4 = bromide, 5 = nitrate, 1.0 mM each.

concentration. On the other hand, if the concentration of the mobile phase was too high, the analytes could not be separated completely. Considering the experimental results, 100 mM of potassium chloride was selected as a mobile phase for the following experiments.

3.3. Analytical Figures of Merit

The RSD of the retention time for the six successive chromatographic run under the optimum condition were in the 1.09% - 1.75% range. The RSD for the retention time were less than 2%. It showed that this method had

good repeatability.

The calibration curves of the five inorganic anions are shown in Figure 5. The calibration graphs showed linear relationships between the peak area and the concentration. It can be seen from the good R-square values obtained.

The limits of detection (LOD) were 0.15, 0.18, 0.14, 0.15, 0.08 mM for iodate, bromate, nitrite, bromide, nitrate, respectively. On the other hand, the limits of quantitation (LOQ) of iodate, bromate, nitrite, bromide, nitrate were 0.50, 0.61, 0.46, 0.49, 0.26 mM, respectively. The values of LOD and LOQ were low enough. It showed that this method had good sensitivity.

3.4. Practical Application

The monolithic column was applied to the determination of inorganic anions present in tap water and ground water samples. The results are shown in Figure 6. There were no inorganic anions identified in the tap water sam

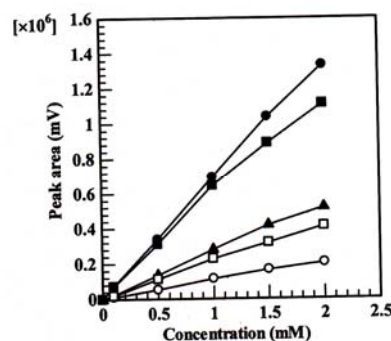


Figure 5. Calibration curves for five inorganic anions. Column, 80×0.32 mm i.d.; mobile phase, 100 mM KCl; flow rate, $4 \mu\text{L}\cdot\text{min}^{-1}$; injection volume: 0.2 μL ; wavelength of UV detection: 210 nm; ▲ = iodate, ○ = bromate, ■ = nitrite, □ = bromide, ● = nitrate.

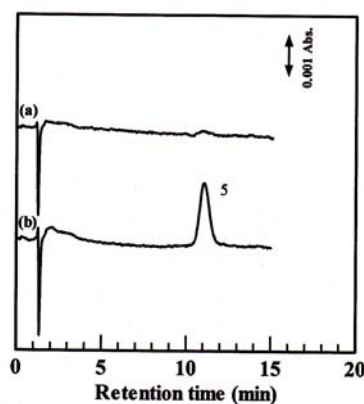


Figure 6. Separation of inorganic anions in water samples. Column dimension: 80×0.32 mm i.d.; mobile phase: 100 mM KCl; flow rate: $4 \mu\text{L}\cdot\text{min}^{-1}$; injection volume: 0.2 μL ; wavelength of UV detection, 210 nm; sample: tap water (a) and ground water samples (b).

ple (Figure 6(a)). On the other hand, nitrate was identified in the ground water sample (Figure 6(b)), with concentration of nitrate is 0.08 mM. The concentration of nitrate as calculated according to the peak area was 0.08 mM.

4. Conclusion

A poly(glycidyl methacrylate-co-ethylene dimethacrylate) anion exchange monolithic column was successfully produced by *in situ* polymerization further modified with trimethylamine via ring-opening reaction of epoxy group. Morphology of the monolithic column was studied by scanning electron microscopy. Mechanical stability and permeability of the column were both good. In general, this method provides good precision of retention time, acceptable linearity and good sensitivity. The present method could be applied to the determination of inorganic anions contained in tap water and ground water with the good values for recovery.

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