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Research Article

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Separation of inorganic anion from biomaterial using methacrylate-based column in ion chromatography capillary system

Hidayat[a], Rahmiana Zein[a]*, Edison Munaf[a], Lee Wah Lim[b] and Toyohide Takeuchi[b]

[a]*Department of Chemistry, Faculty of Mathematic and Natural Science, Andalas University, Padang, Indonesia*
[b]*Department of Chemistry, Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu, Japan*

ABSTRACT

Ion chromatography has widely used to determination of inorganic anions species present in various samples. Polymer methacrylate based monolith column initiated by polymerization of poly (GMA-co-EDMA) has been used for the separation of inorganic anions: IO_3^-, BrO_3^-, NO_2^-, Br^-, NO_3^-. The column was made in the presence of selected porogens and characterized using scanning electron microscopy (SEM). The resulting monolithic column has good performed for the separation of inorganic anions in biomaterial samples (Arenga pinnata, Lima beans, Malay apple, and Sapodilla)

Keywords: ion chromatography, monolithic capillary, methacrylate polymer

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INTRODUCTION

Since Small et al. in 1975 has introduced ion chromatography, it had become a routine analytical method for the determination of inorganic and organic ionic species present in various samples [1]. Analytes can be separated due to differences in their electrostatic interactions with stationary phases packed in a column prior to moving into the detector [2]. Ion chromatography became an interesting method because of the low reagent consumption, rapid and efficient separations, can analyze small volumes of high matrix complexity sample, and simple interfacing [3].

Ion chromatography is the high performance form of ion exchange chromatography. In ion chromatography with suppressed conductivity detection, the separator column effluent passes through a suppressor column which chemically reduces the eluent background conductance, while at the same time increasing the electrical conductance of the analyte ions [4]. Ion chromatography became particularly attractive for the new chromatographic challenges of complex matrices analysis, fast chromatography or on-chip separations because the combination of their singular porous properties, the various chemistries available and their relatively simple implementation in columns with small internal diameters [5].

Monoliths are highly porous inorganic or organic materials originally developed for conventional capillary HPLC separations [6]. Since early 1990s, *polymethacrylate* monoliths has introduction to emerged as a powerful alternative tool in chromatographic column technology. In comparison to packed columns, monoliths enable fast and efficient separations under relatively low pressures, because of its large pores, so greater hydrodynamic flow can be used to increase separation speed [7]. The monolith has unique properties, in particular their tolerance to high flow rates while maintaining excellent peak efficiencies, and the rapid speed of chromatographic separations that can be achieved at acceptable backpressures, make the monolithic column format superior in some applications to the more commonly used packed columns [8]. Monoliths can be readily adapted for different separation mechanisms without further treatments by tuning the prepolymer composition and fabrication process [6].

Three types of monolithic supports are currently available: (1) inorganic polymers based on silica and more recently on carbon and zirconia, (2) synthetic organic polymers such as *polymetha-crylates*, *polyacrylamide*, *polystyrenesdivinyl-benzene* and (3) natural polymers such as *agarose* and *cellulose* [9]. There are two type pores of monolithic material in the structure of the column, *mesopores* and *macropores* (through pores). Based on the classification of IUPAC, *mesopores* have diameter pores smaller than 2 nm, *macropores* have diameter between 2 and 50 nm, and diameter larger than 50 nm for through pores [10]. Polymerization organic polymer monolith column can be prepared with various ways. Some of which are thermally initiated polymerization and photopolymerization using UV light has used to prepared organic polymer monolithic column [11].

This research aims to learn the reaction in the monolith column as the stationary phase, learn the influence of various and concentration of the mobile phase to separation of anions, and then to applying the methods to separation anions on the sample biomaterials.

EXPERIMENTAL SECTION

Materials

Capillary LC system consisting of data processor, UV detector (Jasco Tokyo, Japan), the injector volume 0.2 µL (Rheodyne, Cotati, CA, USA), microfeeder pump (L.TEX Corporation, Tokyo Japan) that use gas-tight syringer (0.5 mL; Ito, Fuji, Japan), capillary column (100 mm x 0.32 mm ID x 0.75 mm OD), PTFE (1/16 mm ID x 0.25 mm OD (GL Science, Tokyo, Japan)), waterbath, oven, syringe 0.5 mL; 0.1 mL; 0.25 mL; (Ito, Fuji, Japan), Scanning Electron Microscopy (SEM) S-4800 (Hitachi) and Fourier Transform Infra Red (FTIR) Irtron IRT-30 (Jasco).

All the reagents used were of analytical grade: 1,4-*butanediol*, $NaNO_2$, NaBr, $NaNO_3$ and $NaIO_3$ (Nacalai Tesque, Kyoto, Japan), 3-*(trimethoxysilyl)-propyl methacrylate* (γ-MAPS) (Trade TCI Mark), decanol, ethanol, methanol, azobisiso-butyronitrile (AIBN), and NaOH (Wako Pure Chemical Industries, Osaka, Japan). IC water from GS-590 water destillation system (Advantec, Tokyo, Jepang).

Monolith column was prepared by *glycidyl methacrylate* (GMA), *ethylene dimethacrylate* (EDMA), *tryethylamine* (TEA). All reagents were obtained from Wako Pure Chemichal Industries, Trade TCI Mark, and Nacalai Tesque.

Biomaterial samples: lima beans and nipah palm. Biomaterials were soaked for 2 hours with NaOH, then filtered with a 0.45µm PTFE membrane filter. Filtrates are then prepared for separation.

Preparation of monolithic column

Methacrylate-based column: prior to polymerization, the capillary column pretreated with the following procedure: first step, the capillary column (0.32 mm i.d x 0.75 mm o.d) with a length 10 cm was rinsed with 1.0 M NaOH, water, 0.1 M HCl with flow rate 4 µL/min for 0.5 h, respectively. The capillary column silanized by filling with a mixture 0.15 mL γ-MAPS in acetone 0.35 mL, sealed both ends capillaries with PTFE and kept in a water bath for 24 h in 60˚C. The capillary column was rinsed with acetone for 0.5 h and dried with nitrogen gas for 0.5 h.

The polymer precursor solution prepared from 0.002 g AIBN (polymerization initiator), 0.09 mL GMA (monomer), 0.03 mL EDMA (cross-linker), 0.105 mL 1,4-butanediol, 0.06 mL decanol, and 0.015 mL etanol (porogen). The solution was mixed ultrasonically for 5 minute to homogenized. After sonication of capillaries was completed, then it is filled with the mixture and sealed both end with PTFE. The sealed capillaries are submerged into water bath for 24 h in 60˚C. After that the capillary column were washed with methanol about 2 h using an HPLC pump to remove unreacted monomers and the porogenic solvent.

Typically, TEA was pumped through the column to completely wet the polymer first, and then the column was sealed at both ends by a closed loop filled with TEA, and heated at 80˚C for 4 h in an oven. Finally, capillary column rinsed with methanol about 0.5 h [10].

Characterization of *Methacrylate-based* Monolith Column

The monolith column was cut with a length of approximately 2-3 mm. Characterization are done with Scanning Electron Microscope (SEM). The monolith coated with precious metal i.e. platinum or a gold/palladium alloy using Sputter 8. The image was recorded from the area at the enlargement remark in SEM which was chosen randomly [10].

RESULTS AND DISCUSSION

Preparation and Characterization of *Methacrylate-based* Monoliths Column

The quality of porogen solvent, composition of monomer and cross-linker, the ratio between monomer and porogen phases, and the polymerization temperature could be influence the morphology of monolithic column [12]. The morphology of monolith column was an important parameter to capability and efficiency separation [13]

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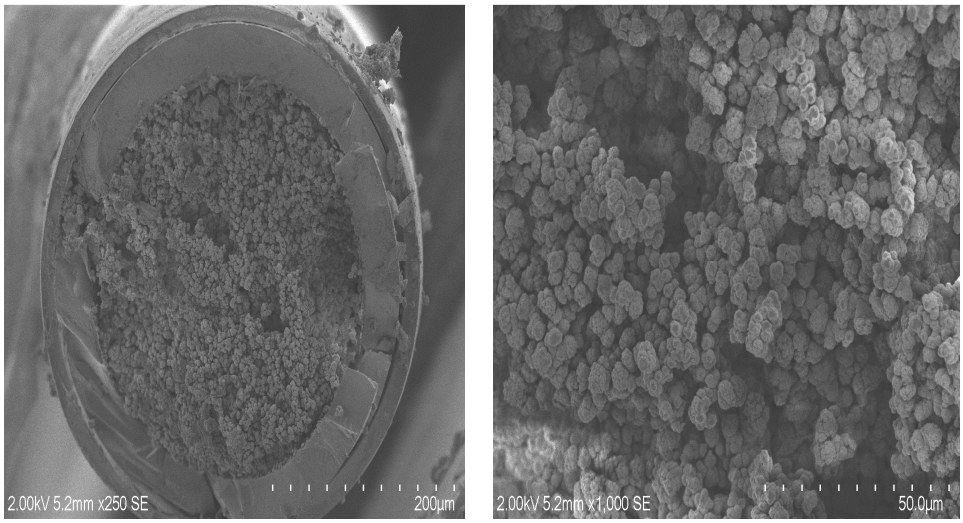

(A) **(B)**

Figure 1. Scanning Electron Microsphotograph of monolith column (A) 250x (B) 1000x

Characterization of monolith column with SEM aims to see of morphology and density of monolith column has been prepared. An SEM image of a monolith column is shown in Figure 1. As can be seen in Figure 1a, the morphology of monolith column was solid and completely attached to the inner of the capillary column. It was influenced by the pre-treatment step if the capillary column. Meanwhile, in Figure 1b has seen numerous clusters of fused globular structure with uniform diameter. The monolith formed by mesopores and trough pores can be seen.

Permeability of Methacrylate-based Monoliths Column

Permeability is an important aspect of monolith performance. Permeability monolithic column was study by injection of the mobile phase onto supporting material formation. Mobile phase were pumped with flow rates from 0.5 to 4.0 µL/min. Permeability based on slopes of backpressure versus flow rate is shown in figure 2. The permeability value was calculates by using Darcy's Law, $B_o = F\eta L/(\pi r^2 \Delta P)$, where F is the flow rate of the mobile phase (m^3/s), η is the viscosity of the mobile phase (Pa.s), L is the effective length of column (m), r is the inner radius of the column (m), and ΔP is the pressure drop of the column (Pa) [14]. The permeability value of a single poly(GMA-co-EDMA) monolithic column are 9.879 x 10^{-13} m^2 with linear relationships, R>0,9997.

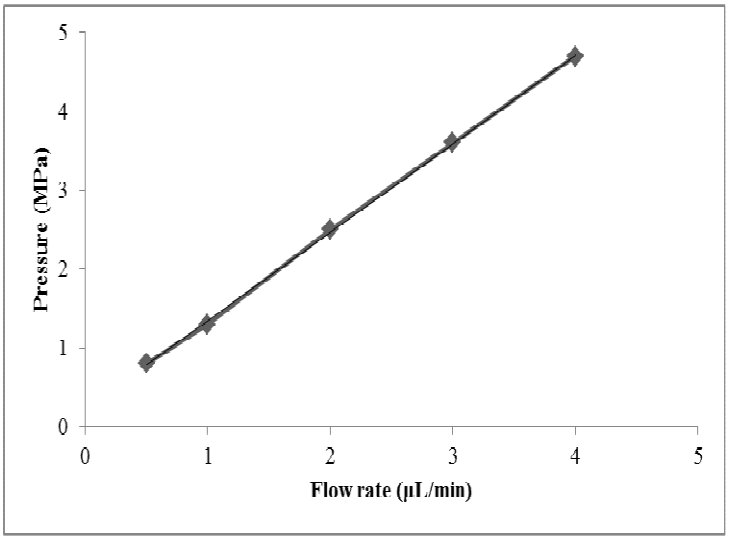

Figure 2. Permeability of the *Methacrylate-based* monolith column

Repeatability and Stability Methacrylate-based Monoliths Column

Repeatability and stability are basic conditions for a monolithic column, especially when the column is constantly used for analysis. Repeatability and stability tested by injection of analyte six times. The result presented in Table 1 shows capillary RSD values below 1.0 %. The RSD results indicated the good run-to-run reproducibility could be easily obtained.

Table 1. Capillary repeatability RSD for retention factor of iodate, bromate, nitrite, bromide, and nitrate for ion chromatography

No	Analite	RSD (%)
1	IO_3^-	0.77
2	BrO_3^-	0.23
3	NO2-	0.30
4	Br-	0.17
5	NO3-	0.05

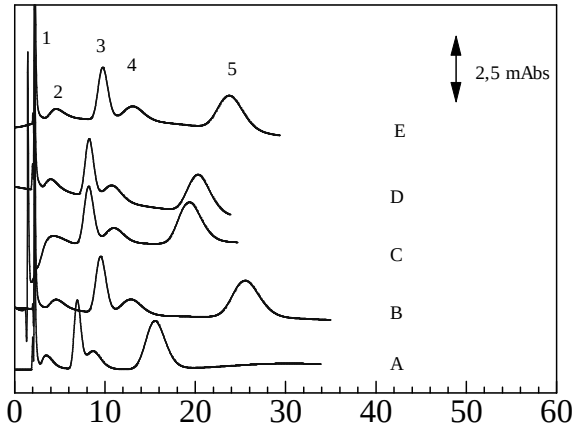

Figure 3. Effect of different kind of mobile phase on the separation of anion. (1) IO_3^-, (2) BrO_3^-, (3) NO_2^-, (4) Br^-, (5) NO_3^-; Column 100 mm x 0.32 mm i.d.; Flow rate 4 µL/min. Mobile phase (A) NaCl (B) LiCl (C) KCl (D) NH_4Cl (E) RbCl

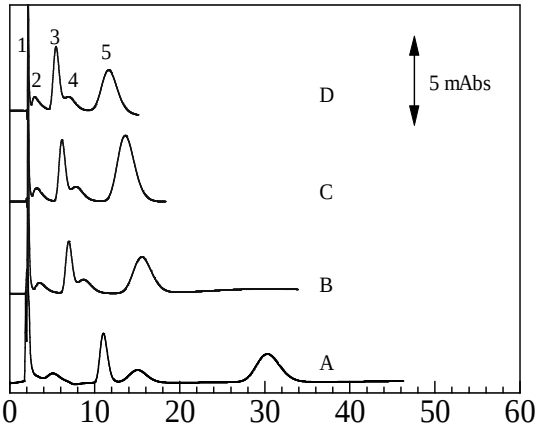

Figure 4. Effect of concentration of sodium chloride mobile phase on the separation of anion. Sodium chloride concentration : (A) 100mM, (B) 200 mM, (C) 300 mM, (D) 400 mM

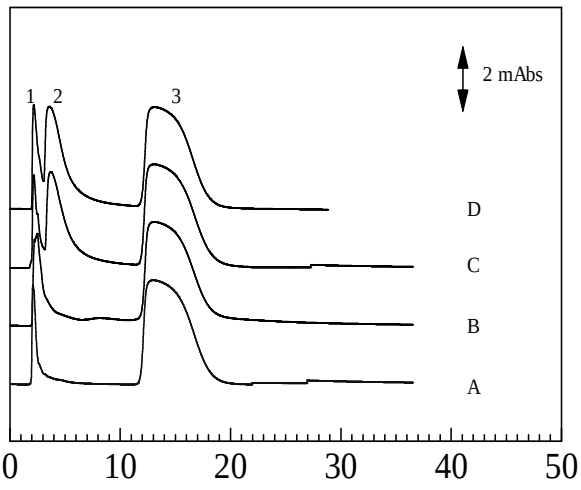

Figure 5. Chromatogram of inorganic anions in biomaterial samples. (1) IO_3^-, (2) BrO_3^-, (3) NO_2^-. Column: 100 mm x 0.32 mm i.d. Flow rate 4 µL/min. Wavelength of UV detection 210 nm. (A) *Sapodilla,* (B) *Malay apple,* (C) *Lima bean,* (D) *Arenga pinnata*

Effect of Eluent on the Separation of Inorganic Anions

Ionic strength, pH and type of anion can influence the eluent strength of mobile phase. Figure 3 shows of the separation inorganic anions on monolith column using NaCl, LiCl KCl, NH_4Cl, and RbCl with concentration 100 mM, respectively, as the mobile phase. NaCl have a shorter retention time and more reproducible signal than other, so it can give the faster separation time. The effect of sodium chloride mobile phase concentration was studied using

various concentration of sodium chloride, i.e., 100, 200, 300 and 400 mM. Figure 4 shows the retention time decreases with increasing sodium chloride concentration, and higher concentration of sodium chloride make the analyte could not be separated completely. Considering the result, NaCl with 100 mM was selected as a mobile phase for the following experiments.

Determination of Anions in Biomaterials Sampel

The monolith column was applied to analyze the anions in *Arenga pinnata, Lima beans, Malay apple, and Sapodilla* samples. Figure 5 shows the separation of anions in biomaterial samples on monolithic column modified with triethylamine. The results indicate that iodate ion was present in all of biomaterial samples with concentration: 0.148 mM; 0.086 mM; 0.069 mM; and 0.055 mM for *Malay apple*, *Arenga pinnata*, *Lima bean*, and *Sapodilla*, respectively. Bromate ion was present in *Arenga pinnata* and *Lima bean* with concentration 0.106 mM and 0.092 mM, respectively, but it was not detected in *Malay apple* and *Sapodilla*. Based on research data and calculation, nitrite on chromatograph was a nitrite derivate from nitric acid solvent used during sample preparation. Meanwhile, bromide and nitrate were not detected in all biomaterial samples.

CONCLUSION

A poly (glycidyl methacrylate-co-ethylene dimethacrylate) anion exchange monolithic column was successfully produced by *in situ* polymerization further modified with trimethylamine. Monolith column were characterized by SEM. Repeatability, stability and permeability of the column were also determined. 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 biomaterial samples.

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