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Research Article

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Separation of inorganic anions from biomaterial samples from Indonesian traditional fruit using monolithic column in ion chromatography capillary system

Hidayat[1], Rahmiana Zein[1], Hermansyah Aziz[1], Edison Munaf[1], Lim Lee Wah[2] and Toyohide Takeuchi[2]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, Indonesia
[2]Department of Chemistry and Biomoleular Science, School of Engineering,Gifu University, Gifu, Japan

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ABSTRACT

Monolith column by in situ polymerization reaction has prepared with using glacidyl methacrylate as monomer, ethylenedimethacrylate as cross-linker, 1,4-butanediol, decanol, and water as (orogeny solvent, azobisiso-butyronitrile (AIBN) as polymerization initiator, and trymethylamine as modifier. The morphology of the monolith column has characterized using Scanning Electron Microscopy (SEM). This method has applied to determination of inorganic anion containing in biomaterial material waste from Indonesian traditinal fruit such asbuah tap (Arengapinnata, jambu bol (Malay apple, and buah saus (Sapodilla).Iodate ions present in each biomaterial sample. The iodate ion which concentration 0.038 mM and 0.069mM for ArengaPinnata, and Sapodilla, respectively.One the other hand,iodate ion undetected in Malay apple.

Keywords: ion chromatography, monolith column, trymethylamine, glacydilmethacrylate, biomaterial, Indonesian fruit.

INTRODUCTION

Ion chromatography has become a routine analytical method for the determination of inorganic and organic ionic species present in various samples since its introduction in 1975 by Small et al. Largely stationary phases used in ion chromatography have charged or chargeable moieties that can interact with ionic analytes through attractive or repulsive electrostatic forces[1]. Ion chromatography has been favorites because of low reagent consumption, rapid and efficient separations, analysis of small sample volumes of high matrix complexity, and simple interfacing[2].

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[3].

Ion chromatography (IC) has developed into a mature and widely used technique for the separation and analysis of a considerable variety of analytes[4].Since their introduction in the early 1990s, polymethacrylate monoliths have emerged as a powerful alternative tool in chromatographic column technology. The combination of their singular porous properties, the various chemistries available and their relatively simple implementation in columns with small internal diameters make them particularly attractive for the new chromatographic challenges of complex matrices analysis, fast chromatography or on-chip separations[5].

There are three types of monolithic, namely inorganic polymers based on silica and more recently on carbon and zirconia, synthetic organic polymers such as polymetha-crylates, polyacrylamide, polystyrenesdivinyl-benzene and

natural polymers such as agarose and cellulose[6]. Polymerization organic polymer monolith column can be prepared with various ways, some of which are thermally initiated polymerization and photopolymerization using UV light.[7].

The greater chromatography efficiency is shown by the monolithic columns for the separation of macromolecules such as nucleotides, oligo-nucleotides, peptides and protein. Therefore, the monolithic columns have been applied in many field, including environmental, pharmaceutical, and genomic applications[8].

In this work, monolithic column prepared by thermal polymerization using glycidyl methacrylate (GMA) as monomer, ethylene dimethacrylate (EDMA) as cross-linker, decanol, water and 1.4-butanediol as porogen, azobisisobutyronitrile (AIBN) as the thermal initiator, and trymethylamine as modifier. The monolithic column using capillary column with a length 10 cm. The morphology of column then characterize with SEM.

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), GS-590 water distillation system (Advantec, Tokyo, Japan), capillary column (100 mm x 0.32 mm i.d x 0.75 mm o.d), PTFE 1/16 mm i.d x 0.25 mm o.d; 0.26 mm i.d x 2 mm o.d; 1 mm i.d x 2 mm o.d; 2 mm i.d x 4 mm o.d (GL Science, Tokyo, Japan), waterbath, oven, syringe 0.5 mL; 0.1 mL; 0.25 mL; 1 mL (Ito, Fuji, Japan), Scanning Electron Microscopy (SEM) S-4800 (Hitachi)

All the reagents used were of analytical grade: Glycidyl methacrylate (GMA), 1,4-butanediol, and trymethylamine (NacalaiTesque, Kyoto, Japan), 3- (trimethoxysilyl)-propyl methacrylate (γ-MAPS) (Trade TCI Mark), Ethylene dimethacrylate(EDMA), 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), $NaNO_2$, NaBr, $NaNO_3$ and $NaIO_3$(NacalaiTesque, Kyoto, Japan). Biomaterial samples: arengapinnata, malay apple,and sapodillasoaked for 2 hours with NaOH, then filtered with a 0.45µm PTFE membrane filter. Filtrate prepared for separation.

Methods

Preparation of monolithic 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 for 0.5h with flow rate 4 µL/min, respectively. After that, the capillary column silanized by filling with 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. Then capillary column rinsed with acetone for 0.5 h and dried with steam nitrogen gas for 0.5 h. The polymer precursor solution prepared from0.002 g AIBN (polymerization initiator), 0.09 mL glycidyl methacrylate (monomer), 0.03 mL ethylene dimethacrylate (cross-linker), 0.105 mL 1,4-butanediol, 0.06 mL decanol, and 0.015 mL water (porogen). The solutions mixed with ultrasonicated for 5 minute to make it homogeneous. Then, capillary column purged with nitrogen gas for 0.5 h. After the pretreated of capillaries was completely, filled with the mixture, and sealed both end capillaries with PTFE. The sealed capillaries submerged into water batch for 24 h in in 60˚C. Then, capillary column rinsed with 0.5 mL methanol with flow rate 4 µL/min. Capillary column treated with 0,5 mL mixture trimethylamine in ethanol (1:1 v/v) with flow rate 0.5 µL/min, heated for 3 h in 80˚C, and rinsed with methanol (flow rate 4 µL/min).

Characterization of Monolith Column

Monolith columns with a length of approximately 2-3 mm were cut from the column which was characterized by scanning electron microscopes (SEM). Coating/ coloring was done using precious metals i.e. platinum or a gold/palladium alloy using Sputter 8. Coating/coloring was done to increase the contrast between the preparations that would be observed and the surrounding environment. The images were recorded from the area at the enlargement remark in SEM which was chosen randomly.

RESULTS AND DISCUSSION

Preparation and Characterization of Polymer Monoliths Column

The morphology of monolith column depending by the quality of the porogen solvent, concentration of the monomer and cross-linker, ratio between the monomer and the porogen phase, and the polymerization temperature[9]. In this research, in situ polymerization has used to prepared the monolith column.

Determination of morphology the monolith column was used Scanning Electron Microscopy (SEM). Figure 1 shows structure of monolith column was solid and completely attached to the inner surface of capillary column. In figure 1b display structure of numerous clusters of fused globular structure with uniform diameter. Mesopores and through pores has formed and forming continues porous channels.

The permeability of monolithic column was examined by measure the back pressure for different flow rate using water as mobile phase. The permeability was calculated by using Darcy's Law[10], $B_o = F\eta L/(\pi r^2 \Delta P)$, where F was the linear velocity of the mobile phase, η was the dynamic viscosity of the mobile phase (η = 0.089 Pa s for water), L was the effective column length, and ΔP was pressure drop.

The flow rate was use in range 0.5 to 4 μL/min. Among of porogen influence the permeability. Good permeability produced makes the mobile phase and sample solution would flow through the column under small backpressure[11]. The permeability of the monolithic column was calculated as 1.566 x 10^{-12}m2 with a correlation coefficient R2 0.99 (Figure 2) which indicated that the monolithic column had good permeability.

Separation of inorganic Anions

The evaluation of performances the monolith column used five anion; (1) iodate, (2) bromate, (3) nitrite, (4) bromide, and (5) nitrate. Figure 3 shows the separation of inorganic anions using various mobile phase. The analytes are detect in wavelength at 210 nm. As seen as in figure 3, sodium chloride provided better eluent to separation anions with a shorter retention time and give a sharp chromatogram peaks.

Meanwhile, to increase the retention time for analyte, sodium chloride with various concentration was examines as the mobile phase. Figure 4 shows sodium chloride with concentration 100 mM give the clearly peak of chromatogram with efficient time. Sodium chloride with concentration more than 100 mM give attached peak of chromatogram, its means analyte not be separation completely and it can complicate the analyte. Figure 4 also shows the retention time of analyte could be increase with the increase mobile phase concentration.

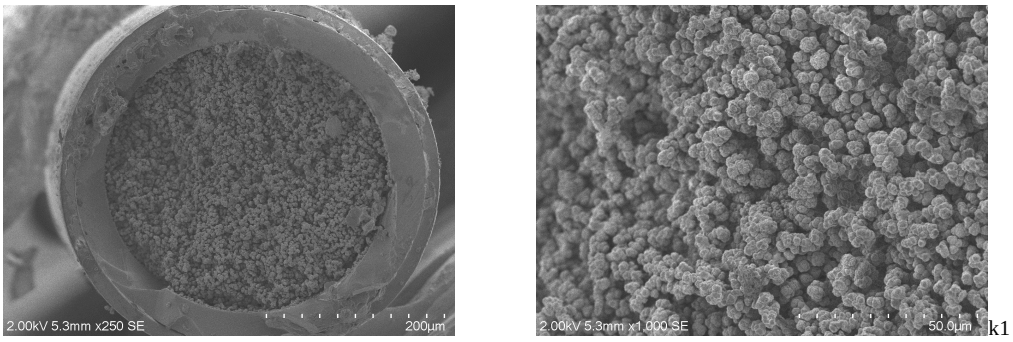

Figure 1. Scanning Electron Microsphotograph of monolith column (a) 250x (b) 1000x

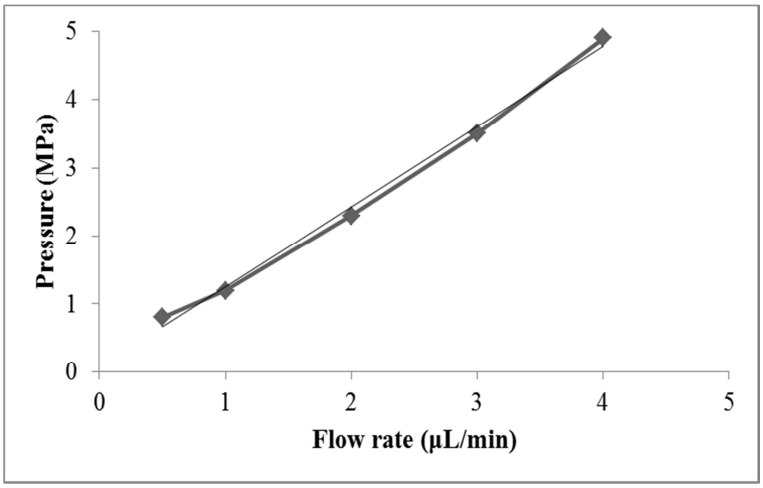

Figure 2. Plots of the floe rate of against the back pressure of the monolith column. Column, 100 x 0.32 mm i.d; mobile phase: water

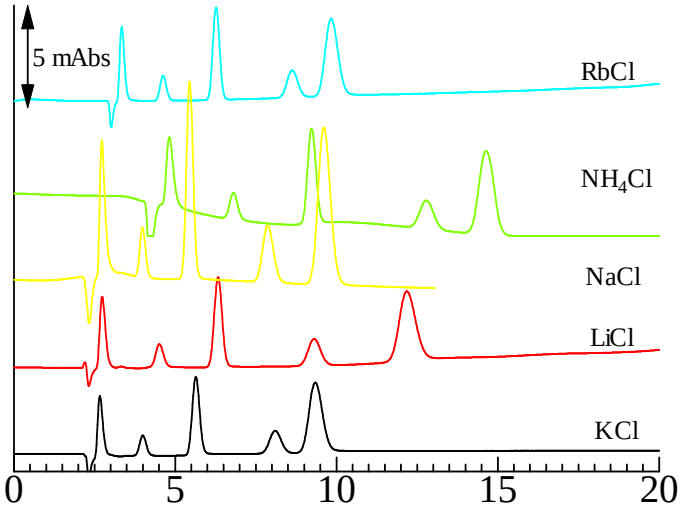

Figure 3. Effect of the mobile phase on the separation of inorganic anions. Column, 100 x 0.32 mm i.d.; mobile phase: (a) KCl, (b) LiCl, (c) NaCl, (d) NH_4Cl, and (e) RbCl; flow rate 4 µL/min; injection volume: 0.2 µL; wavelength of UV detection: 210 nm; analytes: (1) iodate, (2) bromate, (3) nitrite, (4) bromide, and (5)nitrate, 1.0 mM respectively

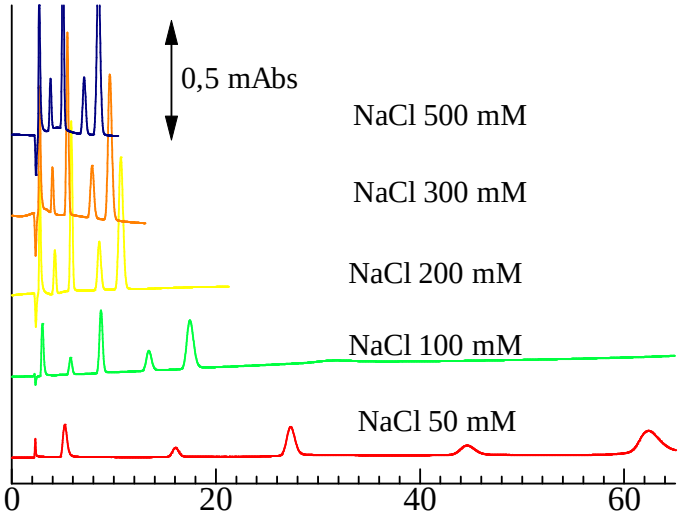

Figure 4. Effect of concentration of sodium chloride mobile phase on the separation of inorganic anions. Column, 100 x 0.32 mm i.d.; concentration of mobile phase: (a) 50 mM, (b) 100 mM, (c) 200 mM, (d) 300 mM, and (e) 500 mM; flow rate 4 µL/min; injection volume: 0.2 µL; wavelength of UV detection: 210 nm; analytes: (1) iodate, (2) bromate, (3) nitrite, (4) bromide, and (5) nitrate, 1.0 mM respectively

Table 1. LOD dan LOQ values of inorganic anions

No	Analyte	LOD	LOQ
1	$IO3^-$	0.017	0.056
2	BrO_3^-	0.021	0.070
3	NO_2^-	0.008	0.026
4	Br^-	0.025	0.084
5	NO_3^-	0.008	0.026

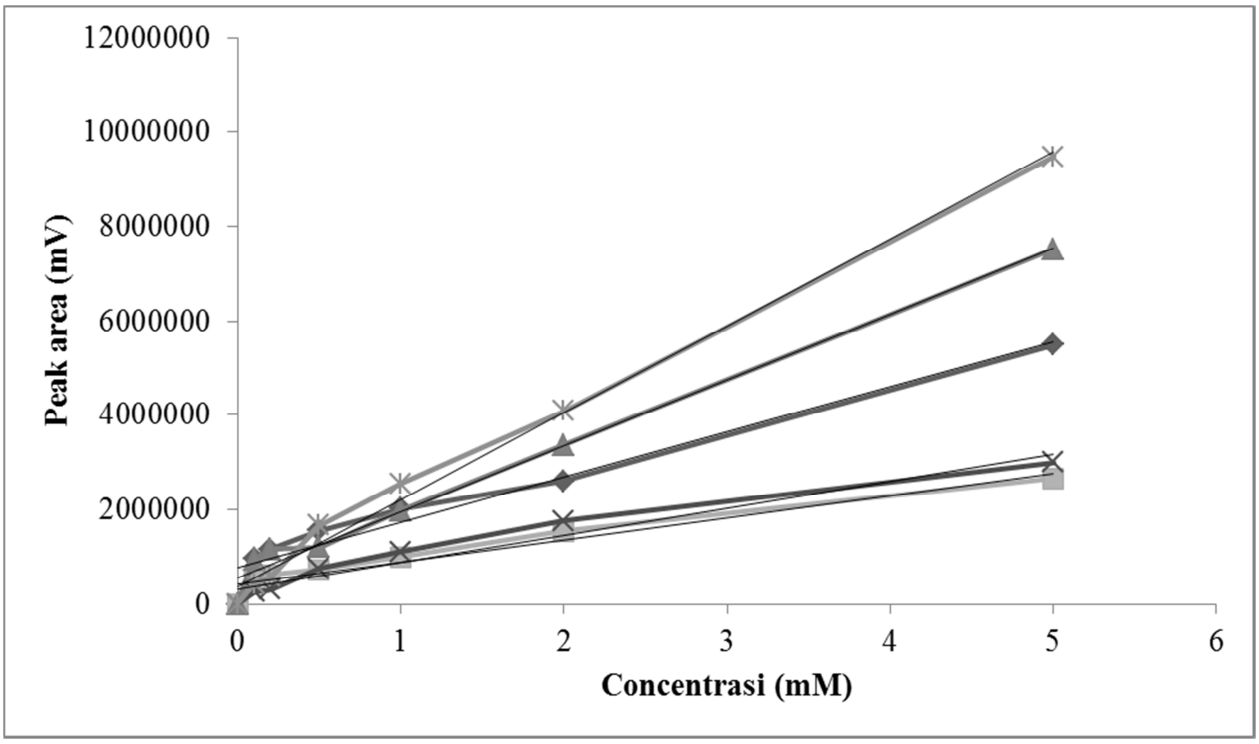

Figure 5. Calibration curves for five inorganic anions. Column, 100 x 0.32 mm i.d; mobile phase: 100 mMNaCl; flow rate: 4 µL/min; injection volume: 0.2 µL; wavelength of UV detection: 210 nm; ♦ = iodate, ■ = bromate, ▲ = nitrite, x = bromide, and * = nitrate

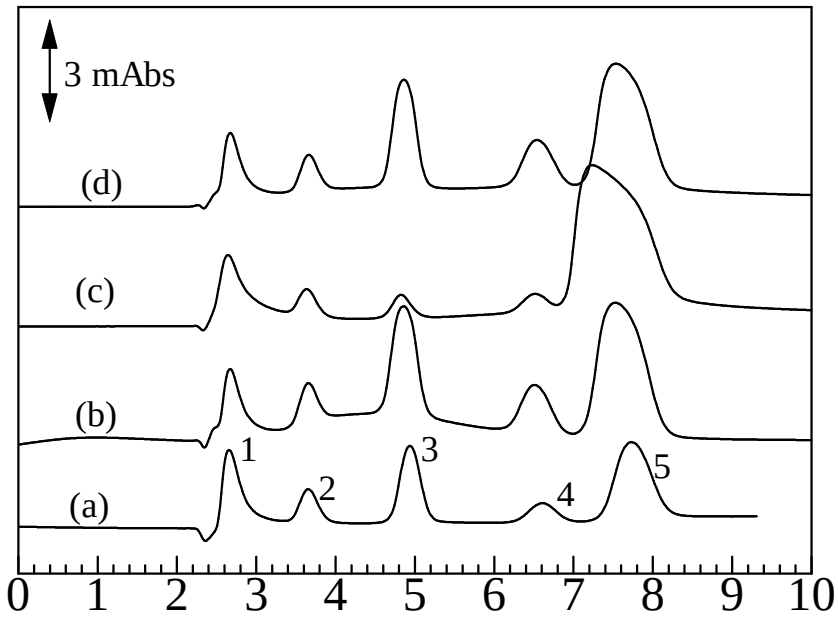

Figure 6.Separation of inorganic anions in biomaterial samples. Column, 80 x 0.32 mm i.d; mobile phase: 100 mMNaCl; flow rate: 4 µL/min; injection volume: 0.2 µL; wavelength of UV detection: 210 nm; samples: (a) Standard solution; (b) ArengaPinnata; (c) MalayApple (d) Sapodilla. Sample: 1. $IO3^{-}$; 2. $BrO3^{-}$; 3. $NO2^{-}$; 4. Br^{-} 5. $NO3^{-}$

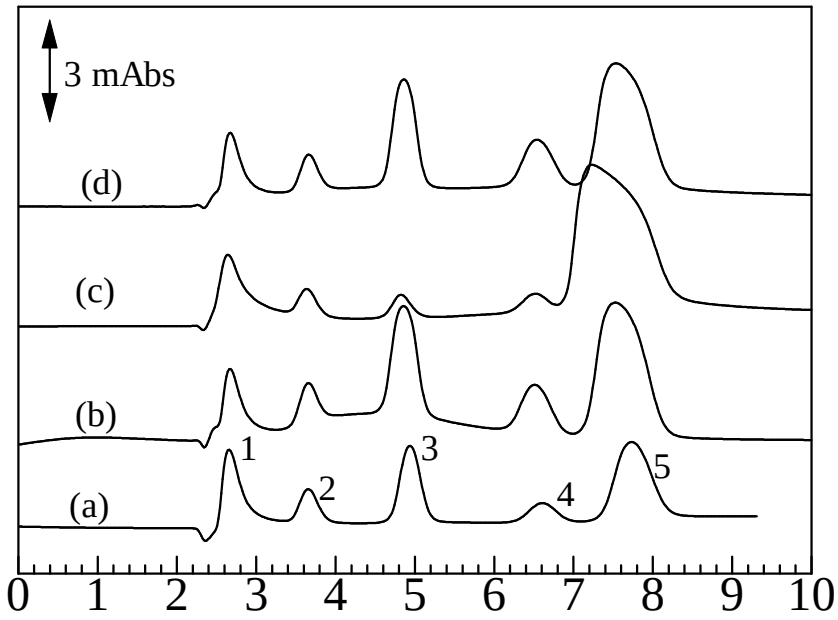

Figure 6.Separation of inorganic anions in biomaterial samples. Column, 80 x 0.32 mm i.d; mobile phase: 100 mMNaCl; flow rate: 4 µL/min; injection volume: 0.2 µL; wavelength of UV detection: 210 nm; samples: (a) Standard solution; (b) ArengaPinnata; (c) MalayApple (d) Sapodilla. Sample: 1. $IO3^{-}$; 2. $BrO3^{-}$; 3. $NO2^{-}$; 4. Br^{-} 5. $NO3^{-}$

Analytical Figures of Merit

The RSD of the retention time for the six successive chromatographic run under the optimum condition were in the 0.104% - 0.657% range. The RSD for the retention time were less than 1%. 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) and limits of quantitation (LOQ) can see in Table 1. The values of LOD and LOQ were low enough. It showed that this method had good sensitivity.

Practical Application

The monolithic column was applied to the determination of inorganic anions present in biomaterial samples. The results are shown in **Figure 6**. Iodate ions present in several biomaterial samples. The iodate ions with concentration 0.038 and 0.069mM for are ngapinnata, and sapodilla, respectively.On the other hand, iodate ions undetected in malay apple.

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