Asian Journal of Chemistry; Vol. 26, No. 12 (2014), 3595-3599

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16536

Preparation and Characterization of Long Alkyl Chain Methacrylate-Based Monolithic Column for Capillary Chromatography in Separation of Alkylbenzene Compound

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Received: 16 October 2013; *Accepted*: 8 January 2014; *Published online*: 5 June 2014; AJC-15300

This research describes preparation and characterization of long alkyl chain methacrylate-base monolith for using as stationary phase in capillary liquid chromatography. Preparation of monolithic column were used dodecylmethacrylate as monomer, ethylene dimethacrylate as crosslinker and used a binary porogenic solvent consist of 1.4-butanediol and other 4 various porogen: ethanol, 1-propanol, isoamyl alcohol and isobutyl alcohol. The monolith column were prepared in 100 × 0.32 mm i.d. capillaries column and were optimized in effect of various mobile phase and effect of concentration of mobile phase for separation alkyl benzene compound. Morphology of monolithic column were characterized using scanning electron microscopy. Permeability of column (K^o) was measured by pumping water through to the column with different linear flow rate. The resulting monolithic column with 1.4-butanediol and 1-propanol as porogen have good performed in separation of alkyl benzene compound with acetonitrile-water (80:20) as mobile phase.

Keywords: Monolithic column, Capillary chromatography, Alkyl benzene.

INTRODUCTION

High performance liquid chromatography (HPLC) have widely use in analysis and separation process. In recent years, HPLC with capillary system has rapid development better than conventional system cause of its low sample and solvent consumption, high detection, high sensitivity and wide variety of analysis sample[1].

In a monolithic column microglobular connected in skeleton resulting monolith with porous structure. Porous structure consist of macropores (throghpores) and mesopores. This porous getting of mobile phase with high flow rate can flow through the stationary phase with low mass transfer resistance and low back pressure than packed column[2-4]. Polymer monolith can consist of inorganic *i.e.*, silica-based monolithic column[5] and organic polymer-based[2,6-8]. The main advantages of organic polymer based compared with silica-based monolith, organic polymer were simple in preparation process, unique efficiency and speed, adaptable for column selectivity[6,9]. Polymerization organic polymer monolith column can be prepared with various ways. Thermally initiated polymerization[10-12] and photopolymerization using UV light[13-15] were used to prepared organic polymer monolithic column. Monolithic column prepared with thermal initiation may have poor homogeneity compared to monolith prepared with UV initiation[16].

Based on monomer organic polymer monolithic column can divide to 4 main groups: (1) styrene-based monolithic column, (2) used of living polymer form a non-polar monolith, (3) methacrylate-based and (4) highly polar acrylamide-based monolith[17]. Organic polymer methacrylate-based has several advantages compared to other organic polymer-based. Methacrylate-based monolith have high stability in wide range of mobile phase pH (2-12), fast and simple preparation, easy to modification especially methacrylate with epoxy functional group and have monomer with wide range polarities[1].

Monolithic capillary column were prepared by radical polymerization of butylmethacrylate (BMA) and ethylene dimethacrylate as crosslinker in presence of ternary porogenic solvent containing 1-propanol, 1.4-butanediol and water[11]. A novel organic polymer monolithic column was prepared using photoinitiated polymerization, bisphenol A dimethacrylate (BPADMA) was selected as a crosslinker and benzyl methacrylate as monomer in presence of a binary porogenic solvent consist of cyclohexanol and 1-decanol[15]. Li *et al.*[8] was prepared a novel porous poly(ethylene glycol) methacrylate-based monolith column by thermally initiated polymerization of poly ethylene glycol methyl ether methacrylate (PEGMEMA) and ethylene dimethacrylate in presence of cyclohexane and cyclohexanol as porogen. However, based on several research, mostly reported preparation organic polymer monolith column using methacrylate-based with short alkyl chain monomers cause of limited solubility of long alkyl chain methacrylate monomer[1,6].

In this research, organic polymer monolithic column were prepared by thermal polymerization using dodecyl methacrylate as monomer and ethylene dimethacrylate as crosslinker in presence of binary porogenic solvent containing 1.4-butanediol made fixed and other various porogen consist of ethanol, 1-propanol, isoamyl alcohol and isobutyl alcohol. Using 100 × 0.32 mm i.d. capillary fused silica column. The morphology of column then characterize with SEM and applied to separation of alkyl benzene compound.

EXPERIMENTAL

1-Propanol and 1.4-butanediol as porogen were supplied by Nacalai Tesque, Inc (Kyoto, Japan). 3-(Trimethoxysilyl)-propylmethacrylate (γ-MAPS) were provided by Trade TCL Mark). Dodecyl methacrylate (DMA) as monomer, ethylene dimethacrylate (EDMA) as crosslinker, 2.2'-azobis isobutyronitrile (AIBN) as initiator and other chemical that use in this research were purchased by Product Wako Pure Chemical Industries (Osaka, Japan). Standard solution alkylbenzene compound at concentration each 0.1 % in acetonitrile.

Capillary LC system equipped with a UV/visible detector (Jasco Corporation, Japan), microfeeder pump (L.TEX Corporation, Tokyo, Japan) that use gas-tight syringer (0.5 mL; Ito, Fuji, Japan), a 6-way switching valve with injection volume 0.2 µL (Upchurch Scientific, Oak Harbour, WA, USA). Capillary monolithic column were prepared in a fused-silica capillary tube (100 mm, 0.32 mm I.D. 0.45 mm O.D) and fused silica tube (50 µm I.D) as flow cell connected to detector were purchased by GL Science (Tokyo, Japan). The capillary column were tested under mobile phase flow rate 3 µL/min.

Preparation of monolithic column: To activated inner surface of capillary column, fused silica capillary tube were prepared by steps of column pre-treatment or silanization. Normally methacrylate silane was used to anchor the polymer monolith on the capillary wall, because it consist of functional group with double bond, which can react with organic polymer and silanol groups on the glass surface[18]. Without anchoring the capillary wall, the polymer cannot withstand with high pressure[19,20]. First step capillary column were flushed with NaOH 1 M solution for 0.5 h and drained with IC water for 0.5 h and flushed with HCl 1 M for 0.5 min with flow rate each step 4 µL/min. After that capillary column were silanized with 3-(trimethoxysilyl)-propylmethacrylate (γ-MAPS) 30 % in acetone and keep in waterbath at 60 °C for 24 h. Then flush with acetone for 0.5 h and dried with stream nitrogen gas for 0.5 h.

Monomer solution and porogen solvent were sonicated for 15 min to mixed into a homogenous polymer mixture. The monomer solution filled to capillary column and both ends were sealed with PTFE septum. The polymerization was performed at 85 °C for 15 min. After polymerization capillary column were flushed with methanol to remove unreacted reagent in column. The resulting column then cut into 100 mm and connected to LC system.

Separation of alkylbenzene compounds: To investigating of retention properties the monolith column from dodecylmethacrylate-co-ethylene dimethacrylate the column were connected to LC system use UV detector with wavelength 254 nm. The mobile phase was acetonitrile/water mixture and samples was a mixture of alkylbenzene compound (*n*-butylbenzene, *n*-hexylbenzene and *n*-octylbenzene) dissolved 0.1 % each in acetonitrile. The experiment was carried out with flow rate 3 µL/min and sample injection was 0.2 µL at room temperature.

Effect of various porogen: In this research we use two kind of porogen. For preparation of polymer monolithic column was prepared with fixed 1.4-butanediol and various other porogen with ethanol, 1-propanol, isoamyl alcohol and isobutyl alcohol.

Characterization of monolithic column: Morphology and pore properties of monolithic column were studied by scanning electron microscopy 4800 (Hitachi, Japan). After analysis analyte were finished, the column were washed and cut in to small pieces and then sample column were coated with platinum.

Effect of various mobile phase: For investigating effect of mobile phase for separation of alkylbenzene compound were used three kind of mobile phase, acetonitrile, methanol and ethanol with optimum concentration that we get from effect of mobile phase concentration.

Effect of mobile phase concentration: Various acetonitrile/water concentrations were prepared to investigate the effect of mobile phase concentration to monolithic column. From this investigation we obtained a plot of mobile phase concentration and retention time. The measurements were performed using the same to LC system.

RESULTS AND DISCUSSION

Selection of porogenic solvent has important role for preparation of monolith column. Altering the porogen only affected on the porous structure of polymer monolith[21-23]. In this research were studied about effect of various alcohol porogenic solvent with different alkyl chain. Preparation of monolith column were used 1.4-butanediol as poor solvent that leads to form a large pore size and use 4 kind of solvent: ethanol, 1-propanol, isoamyl alcohol and isobutyl alcohol as shown in Table-1.

Based on Fig. 1 column (a) and column (b) have no significant difference performance in separation of alkyl benzene compound. Compared with column (c) were use 1-propanol as porogen have a good peak performed in separation of alkyl benzene compound better than column (a) and (b) with retention time more longer than column (a) and (b). If we compared with more short chain solvent at column (d) that use ethanol as porogen, it give the retention time more short than the three column before. This case indicated that with the same composition the porogen solvent with short chain alcohol will have fast separation compare the long chain alcohol compound. And solvent with branch groups chain will give-short retention time compare to normal chain compound. Based on the ability

TABLE-1
COMPOSITION (% v/v) OF MONOMER, CROSSLINKER AND POROGEN IN POLYMERIZATION MIXTURES USED FOR THE PREPARATION OF CAPILLARY MONOLITH COLUMN. DODECYL METHACRYLATE = 24.48 % v/v, ETHYLENE DIMETHACRYLATE = 6.12% v/v, POROGEN 1 = 23.46 % v/v AND POROGEN 2 = 45.92 % v/v

Column	Monomer	Crosslinker	Porogen	
a	Dodecyl methacrylate	Ethylene dimethacrylate	1.4-butanediol	Isobutyl alcohol
b	Dodecyl methacrylate	Ethylene dimethacrylate	1.4-butanediol	Isoamyl alcohol
c	Dodecyl methacrylate	Ethylene dimethacrylate	1.4-butanediol	1-propanol
d	Dodecyl methacrylate	Ethylene dimethacrylate	1.4-butanediol	Ethanol

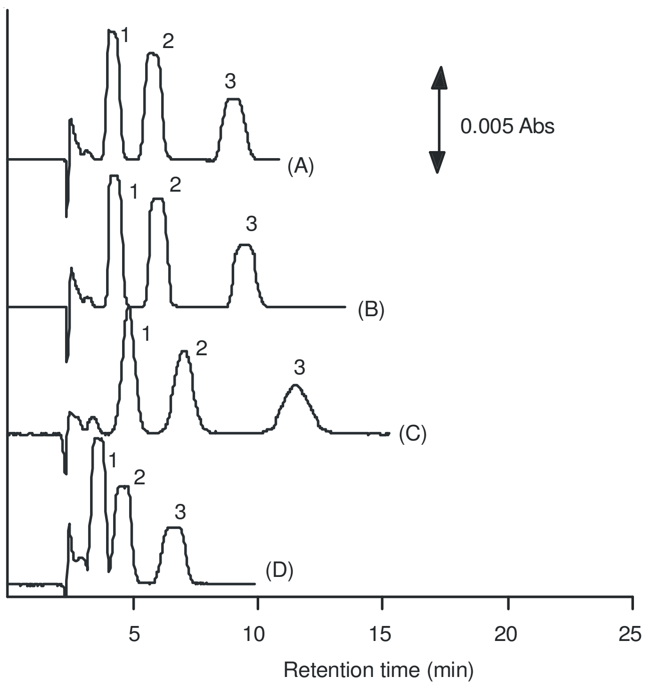

Fig. 1. Chromatogram of separation alkyl benzene compound (1) *n*-butylbenzene, (2) *n*-hexylbenzene, and (3) *n*-octylbenzene with different porogen (a) isobutyl alcohol, (b) isoamyl alcohol, (c) 1-propanol and (d) ethanol. Column : 100 mm × 0.32 mm i.d. Eluent: ACN 80 %, Flow rate: 3 μL/min. Wavelength of UV detection 254 nm

to separation of alkyl benzene compound from fourth column column (c) have a good separation.

Column permeability: An important aspect of monolith performance is its column permeability, which characterized the flow resistance of the chromatographic column[21] was determined by Darcy equation, eqn. (1):

$$K^{o} = \frac{V \cdot \eta \cdot L}{\Delta P \cdot \pi r^{2}}$$

V is the mobile phase linear flow rate, η is the mobile phase dynamic viscosity, ΔP is pressure drop across the column, L is the column length and r is the column inner radius. The calculate K for columns is around 1.863×10^{-11} - 3.711×10^{-11} m^2 (column (a) - column (d)). Normally a separation on monolith column have permeability around 19×10^{-13} m^2 (approximate 8 MPa at 500 nL/min)[12]. Fig. 2 shows the effect of the flow rate through column (a) to (d) on back pressure with water as mobile phase. On column (c) the good linear response between back pressure and flow rate indicated that the column were mechanically stable.

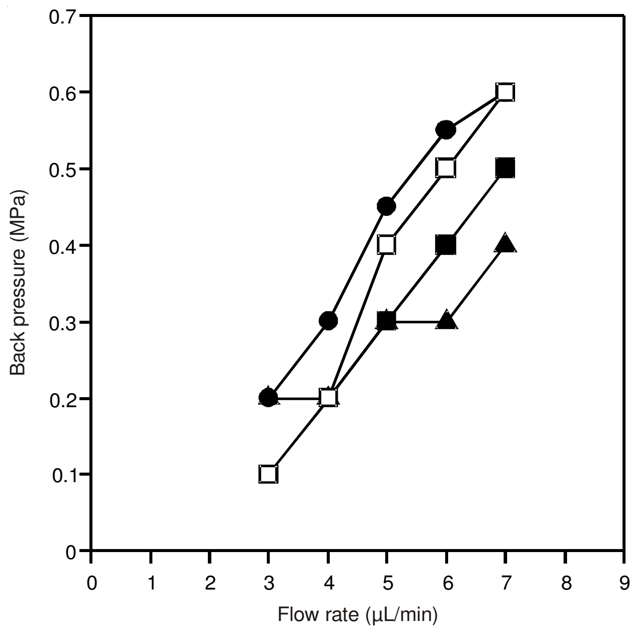

Fig. 2. Back pressure on monolith column as a function of linear flow velocities using water as mobile phase. Permeabilities for these monolith column were calculate to be: 1.863×10^{-11} m^2 (a), 1.484×10^{-11} m^2 (b), 1.863×10^{-11} m^2 (c), 3.711×10^{-11} m^2 (d); ● column a, ▲ column b, ■ column c, □ column d

Reproducibility of monolith column: In addition to good chromatographic performance, reproducibility and stability are basic requirements for a monolithic column, especially when the column is to be used for routine analysis. Reproducibility of all monolithic column was determined through the percent of relative standard deviation (% RSD) for retention time of the three test compounds, *n*-butyl benzene, *n*-hexyl benzene and *n*-octyl benzene. The run-to-run reproducibilities (n = 6) of the each test compounds can see in Table-2. From % RSD confirm that the monolithic column have a good stability and reproducibility.

TABEL-2
COMPARISON % RSD OF FOUR MONOLITH COLUMN BASED ON RETENTION TIME

Column	*n*-butylbenzene (%)	*n*-hexylbenzene (%)	*n*- octylbenzene (%)
a	0.811	1.325	0.504
b	0.281	0.346	0.401
c	0.688	0.591	0.264
d	0.465	2.063	1.693

Morphologies of monolith: The morphology of monolith is depend on composition of monomer and crosslinker, temperature and quality of porogen[17]. The selection of porogen is widely used to controlling the porous properties of monolith without change yhe composition of monomer. Figs. 3 and Fig. 4 shows SEM images of monolithic column with four different porogen solvent. From the SEM image, we see that monolith (c) have more big skeleton size compare with

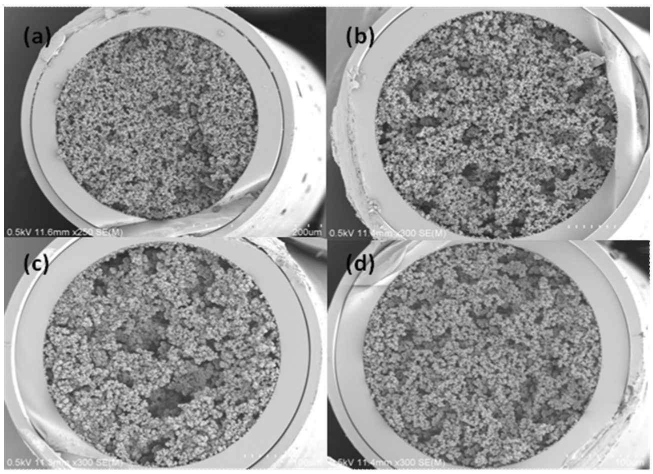

Fig. 3. SEM image of monolith column with different porogen, (a) isobutyl alcohol, (b) isoamyl alcohol, (c) 1-propanol and (d) ethanol

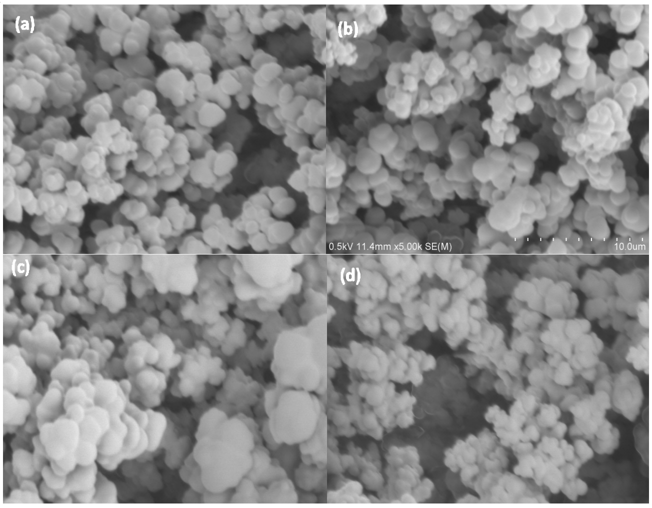

Fig. 4. Bulk region SEM image of monolith column with different porogen, (a) isobutyl alcohol, (b) isoamyl alcohol, (c) 1-propanol and (d) ethanol

other monolith. The skeleton size is about 1-5 µm. Monolith (d) with small molecule solvent compound give more smaller skeleton size, about 1 µm with the bigger through pores. This related with the monolith permeability column (d) that more higher than others. This case means preparation of monolith column using small molecules porogen solvent increase the size of macropores (through pores) an otherwise form smaller skeleton size in polymer monolith.

Optimize separation of monolith column: To optimize separation of monolithic column we studied about effect of various mobile phase and effect of concentration of mobile phase. Several mobile phase were used for separation of alkyl benzene compound, acetonitrile-water, methanol-water and ethanol-water each concentration 80 %. Fig. 5 shows that acetonitrile-water 80 % give the best mobile phase for separation. To determine the best concentration of mobile phase we use several concentration of acetonitrile-water. Fig. 6 shows plot of concentration of mobile phase *versus* retention time using acetnitrile-water as mobile phase for separation of alkylbenzene compound. From that plot we conclude that concentration of acetonitrile-water 80 and 90 % can separate the alkyl benzene compound with short retention time. When we used

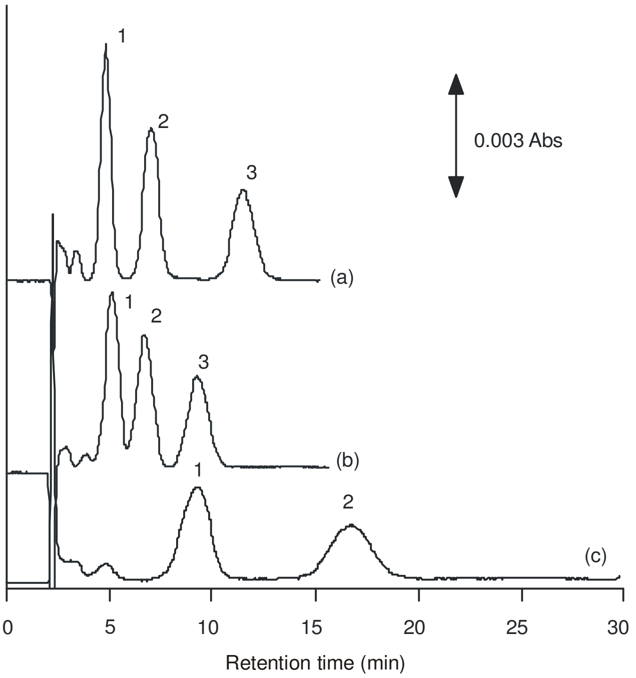

Fig. 5. Chromatogram of separation alkyl benzene compound using 1-propanol as porogen with different kind of mobile phase, (a) ACN-water (80:20), (b) Ethanol-water (80:20) and (c) Ethanol (80:20). (1) *n*-butylbenzene, (2) *n*-hexylbenzene, (3) *n*-octylbenzene. Column: 100 mm × 0.32 mm i.d. Flow rate: 3 µL/min. Wavelength of UV detection 254 nm

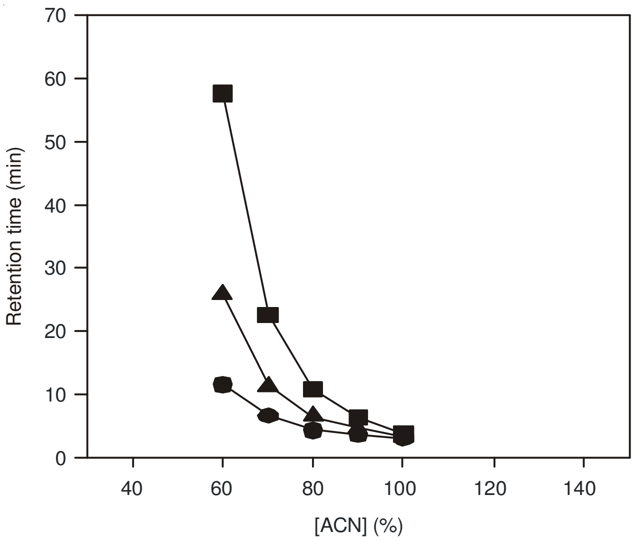

Fig. 6. Graph illustrating plot of concentration of mobile phase *vs*. retention time using acetnitrile-water as mobile phase for separation of alkylbenzene compound; ■ *n*-octylbenzene, ▲ *n*-hexylbenzene, ● *n*-butylbenzene. Flow rate: 3 µL/min

both of concentration of acetonitrile-water for analysis the alkyl benzene compound, mobile phase acetonitrile-water 80 % give the good performance better than acetonitrile-water 90 % (Fig. 7).

Conclusion

A porous organic polymer monolithic column was prepared using dodecyl methacrylate as monomer and ethylene dimethacrylate as crosslinker with variation of porogen by thermally polymerization. The performance of each monolith column was also evaluated. The prepared monolithic column-use

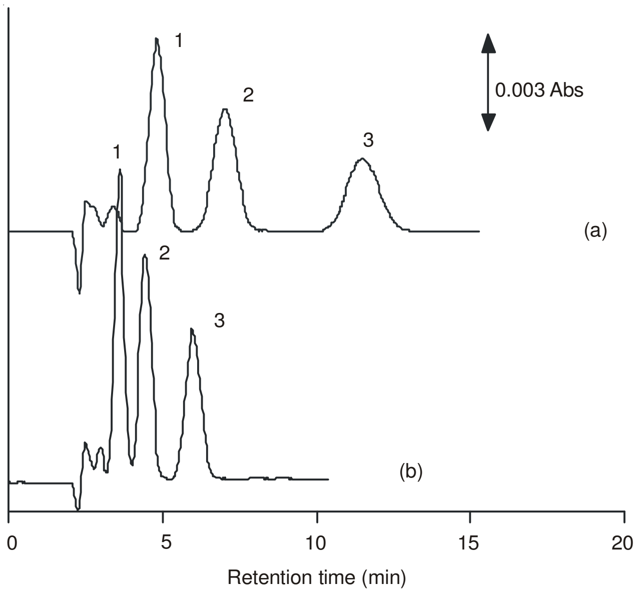

Fig. 7. Chromatogram of effect of mobile phase concentration for separation alkyl benzene compound using 1-propanol as porogen.(a) ACN-water (80:20) and (b) ACN-water (90:10). (1) n-butylbenzene, (2) n-hexylbenzene, (3) n-octylbenzene. Column: 100mm x 0.32mm i.d. Flow rate: 3μL/min. Wavelength of UV detection 254 nm

1,4-butanediol and 1-propanol as porogen give a good performance in separation of alkylbenzene compound. Monolith column were characterized by SEM and permeability and reproducibility were also determined. Furthermore research, still need to study about ratio and composition of various porogent in mixture of polymer monolithic column.

ACKNOWLEDGEMENTS

The authors gratefully acknowledged the Japan Student Service Organization (JASSO) for the scholarship Special Research Student in Gifu University. Thanks are also due to Faculty of Engineering, Gifu University, Japan and Faculty of Mathematics and Natural Science, Andalas University, Indonesia, for their generous support.

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