Preparation and utilization of monolithic column as HPLC stationary phase for alkyl benzene separation with low mobile phase usage

Cite as: AIP Conference Proceedings **2049**, 020006 (2018); https://doi.org/10.1063/1.5082411
Published Online: 14 December 2018

Stevin Carolius Angga, Dias Septiana, Suci Amalia, Warsito, Elvina Dhiaul Iftitah, and Akhmad Sabarudin

Preparation and Utilization of Monolithic Column as HPLC Stationary Phase for Alkyl Benzene Separation with Low Mobile Phase Usage

Stevin Carolius Angga[1,2,3a)], Dias Septiana[1,2], Suci Amalia[1,2], Warsito[1,2], Elvina Dhiaul Iftitah[1,2,b)], Akhmad Sabarudin[1,3,c)]

[1)] *Department of Chemistry, Faculty of Mathematics and Natural Science, Brawijaya University (UB), Malang 65145, Indonesia*
[2)] *Essential Oil Institute (IA), Brawijaya University (UB), Malang 65145, Indonesia*
[3)] *Research Center for Advanced System and Material Technology (ASMAT), Brawijaya University (UB), Malang 65145, Indonesia*

[a)] Corresponding Author : stevin.carolius.angga@protonmail.com
[b)] vin_iftitah@yahoo.com
[c)]sabarjpn@gmail.com

Abstract

One of the long-known separation methods is chromatography, such as high performance liquid chromatography (HPLC). This separation essentially uses column containing particle-packed which generates high flow-resistance and low mass transfer. As matter of fact, it has an impact to high amount mobile phase usage. Meanwhile, organic polymer monolithic column has been widely used as alternative due to its high mass transfer. In addition, the resulting column has stability over wide pH range, high temperature, shrinkage and swelling of the reservoir. The aim of this research was to produce monolithic column which able to utilized for separation with low amount of mobile phase usage. Preparation of monolithic column begins with pretreatment of polyetereterketone tubing column inner wall. It conducted by activating the inner wall with H_2SO_4 49% (v/v), followed by vinylization with glycidyl methacrylate. Furthermore, pretreated column filled by polymer mixture consisted of 30 wt% of monomers glycidyl methacrylate:trimetilolpropane trimethacrylate 4:1 (w/v), 70 wt% of pore-forming agents (1-propanol/1,4-butanediol/water 7:4:1 w/v), and α,α'-azoisobutyronitrile 1 wt% of total monomers amount. The polymerization conducted at 60 °C for 12 h. Produced column connected to HPLC then applied to separate toluene and amylbenzene. The result shows both compounds successfully separated by 7.5 μL/min flow rate of acetonitrile:water (75:25) at 84 and 132 min respectively.

INTRODUCTION

Nowadays, the use of natural product as complementary therapies with medicinal consumption has draw many attentions. Essential oil has been used as fragrance because its volatile properties especially as aromatherapy. Local residents gather the oil by distillation or boiling the natural materials. Even today essential oils have been sold commercially in various forms such as wax and balsam [1-2].

Some studies concerning essential oils have reported the contents in essential oils. Certain compounds contained in these oils turn out to possess variety of important activities. Citronellal from *Cymbopogon nardus* as anti-inflammatory [3], thymol contained in *Thymus vulgaris* L. as anti-microbial [4], and eucalyptus oil (*Eucalyptus citriodora*) may even be used as a pesticide [5].

Although essential oils have various activities that can be utilized, several essential oils contain certain alkyl benzene with negative effects. For instance essential oil from olibanum-tree (*Boswellia sacra*) which induces human

The 3rd International Seminar on Chemistry
AIP Conf. Proc. 2049, 020006-1–020006-6; https://doi.org/10.1063/1.5082411
Published by AIP Publishing. 978-0-7354-1775-5/$30.00

pancreatic cancer cell death also contains toluene (methyl benzene) [6] or wild celery (*Kelussia odoratissima*) as treatment of hypertension and inflammation contains amylbenzene (pentyl benzene). The toluene itself may cause headache, nausea, tinnitus, ataxia, and tremors [6–8]. Meanwhile the amylbenzene may harm aquatic life with long lasting effects [9]. It shows the activities of essential oils may be dangerous when certain compounds as mentioned were not separated firstly. It is important to produce materials for separation which could interact and hold alkyl benzene such as toluene and amylbenzene, with the result that another compounds in essential oil may emitted firstly. Wherein, material must be able to interacts with toluene and amylbenzene. It may be indicated by material performance to separates a solution containing of both compounds.

One of the long-known separation methods is chromatography such as high performance liquid chromatography (HPLC). This separation essentially uses column containing narrow beads that interact with the samples, conforming their properties as to separate the compounds by emitting them in sequence. However, the use of this column is generally limited to certain pH and temperature, it also requires a long separation process which impact to high amount of mobile phase usage [10-11].

Current research on separation developed column using organic polymer monolith. This column is made by organic monomer such as methacrylate using porogen that produces porous monolith with the result of movement of the sample and the separation process become faster with stability over a wide pH range and high temperature. In addition, it could be used for biomolecules with larger size [12-15]. This study aimed to produces monolithic column based on methacrylate which could interact with toluene and amyl benzene which separates a solution containing both of it.

MATERIALS AND METHODS

Chemicals and Materials

All chemicals purchased from different sources were used without further purification. H_2SO_4, acetone, ethanol were purchased from Smart Lab Indonesia. Glycidyl methacrylate (GMA), 1-propanol, and 1,4-butanediol were purchased from Sigma-Aldrich (Singapore). Trimethylolpropane trimethacrylate (TMPTMA), toluene, and amylbenzene from Tokyo Chemical Industry (Japan). Meanwhile, acetonitrile (ACN) from Merck (Indonesia), α,α'-azobisisobutyronitrile (AIBN) from Himedia, and polyetereterketon tubing (PEEK) as column housing (1.00 mm i.d. and 1/16" o.d.) was purchased from Supelco (Canada).

All LC experiments were performed using HPLC unit Prominence 20 from Shimadzu (Japan) equipped with Shimadzu's workstation for system control and data acquisition. The system was composed by communication bus module (CBM-20A), HPLC pump (LC-20AD), UV/Vis detector (SPD-20A), and Rheodyne 8125 injector with custom-made 2 μL PEEK sample loop. Meanwhile, the morphology of monolith was analysed with SEM (FEI Inspect-S50).

Methods

The procedures consisted by few steps, which are pretreatment of PEEK as column housing, preparation of monolith column, study of monolith columns mechanical stability, and finally its utilization for separations of toluene and amylbenzene.

Pretreatment of PEEK

Pretreatment of PEEK was conducted through sulfonation and methacrylation similarly described by Shu et al. [16] with minor modification. The PEEK was filled with H_2SO_4 49% (v/v) and placed at room temperature for 6 h to conduct sulfonation of PEEK. After rinsed it with water, it was methacrylated with 1M GMA in acetone and placed at 60 °C for 4 h. Finally, inside of the column was washed with acetone and cut into 10 cm long leaving the column with reactive alkene groups, which serve as anchorage of monolith to the inner surface during the polymerization. Later on, the succession of pretreatment indicated by the anchoraged of monolit inside column housing even though it was pumped with mobile phase.

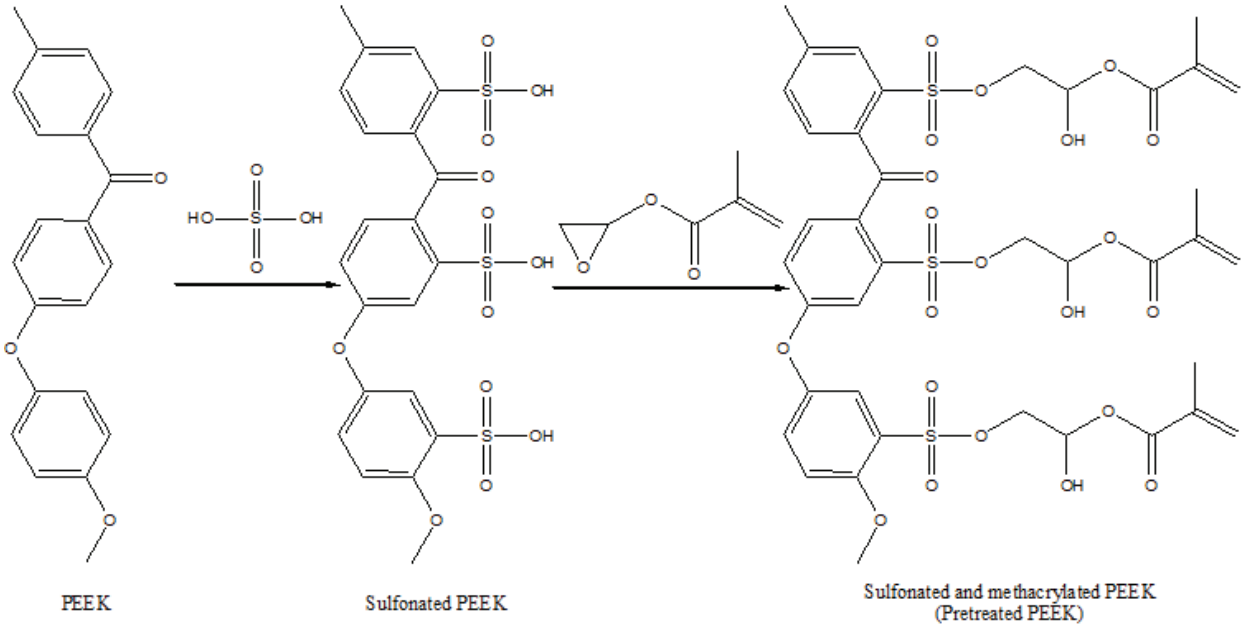

FIGURE 1. Pretreatment of PEEK as column housing (Shu et al, 2012)

Preparation of Monolith Column

The monolith columns was prepared by in situ copolymerization procedure as described by Sabarudin et al. [17] also with minor modification. A monomer mixtures were prepared inside microtube which consisted of 240 µL GMA, 60 µL TMPTMA, and ternary porogens 408 µL 1-propanol, 233 µL 1,4-butanediol, 58 µL water, in the presence of 3 mg AIBN as radical intiator. The mixture was homogenized before filling it into the pretreated PEEK, and polymerization was allowed to continue at 60 °C for 12 h. Subsequently, the obtained monolith column was washed with ethanol and water for 6 h at 10 µL/min respectively to remove unreacted monomers and remaining porogens.

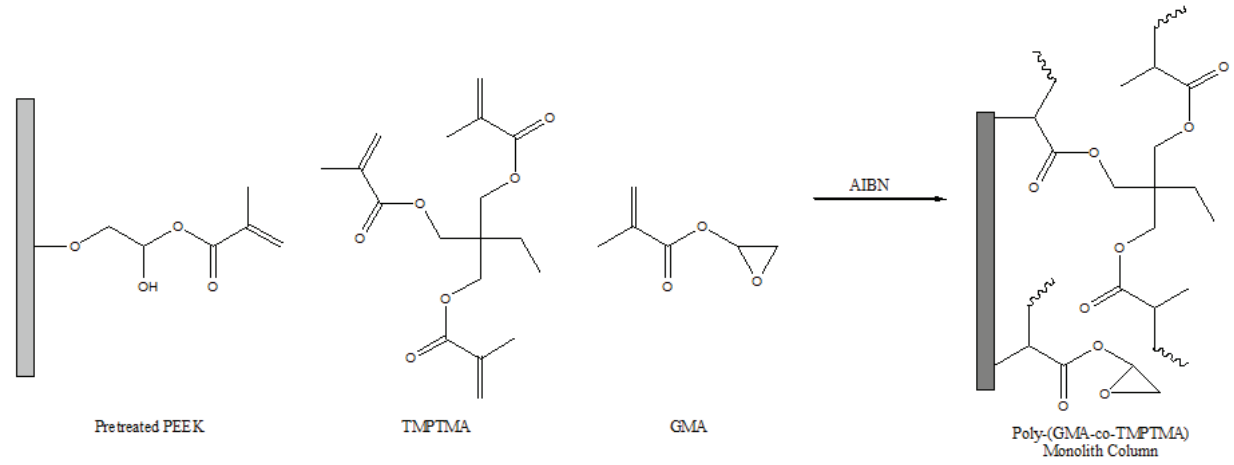

FIGURE 2. Preparation of monolith column (Sabarudin et al, 2012)

Study of Monolith Column Mechanical Stability and Morphology

Produced monolith column mechanical stability was then studied by connecting it into HPLC. It was was pumped at 1-10 µL/min flowrate with 1 µL/min interval. Its mechanical stability was studied from linear regression of each flowrate versus the backpressure resulted. Later on, its morphology was also studied with SEM.

The produced column was then installed to HPLC system and pumped with ACN:water (75:25) at 7.5 µL/min with UV absorption wavelength at 214 nm. Later on, a solution consisted of 25 ppm toluene and 75 ppm of amylbenzene was then injected to demonstrate produced monolith column performance for separations.

RESULTS AND DISCUSSION

Preparation of Monolith Column

Monolith was produced through in situ copolymerization by bonding each alkene group of pretreated PEEK, monomers GMA, and TMPTMA in the presence of radical initiator AIBN. Meanwhile, the porogen (pore-forming agent) which are 1-propanol, 1,4-butanediol, and water play a role to forming space between the monomers during polymerization. After the polymerization conducted, the space becomes flow-through channel for the sample to moves quickly but still be able to interact with the monolith. In addition, large flow-through channel even allows larger molecules to let through.

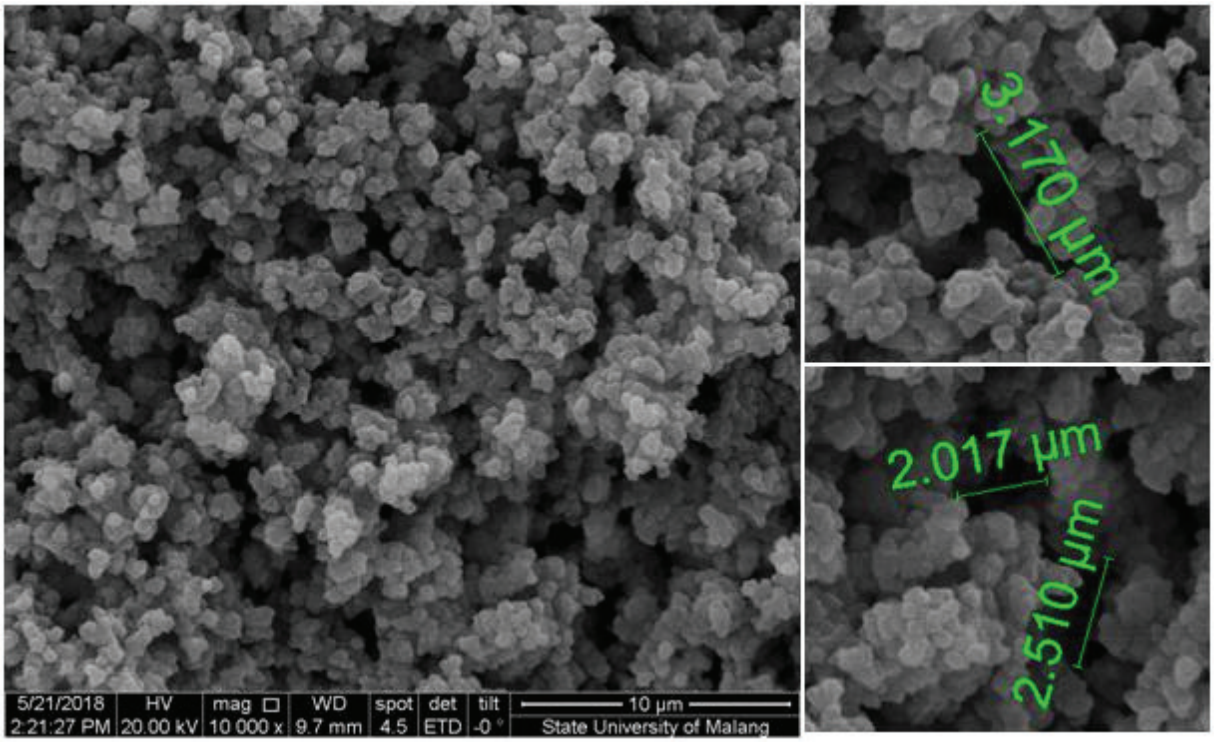

FIGURE 3. Morphology of monolith at 10,000x magnification

As in Fig. 3, at magnification of 10,000x shows the monolith were produced and spread evenly. Flow-through channels formed at 2.0-3.2 µm which allows faster movement of the samples. Moreover, evenly spreadment of the monolith surface still allows the samples to interact with the the monolith in the course of separating process.

Later on, the mechanical stability of monolith column was then studied. It was conducted by pumping ethanol at 1-10 µL/min flowrate with 1 µL/min interval while observe the backpressure resulted. As in Fig. 4, the linear dependence of monolith column backpressure on flowrate shows good mechanical stability to reached $R^2 = 0.9971$. In addition, it also means the monolith column has high sustainibility to be used frequently [18].

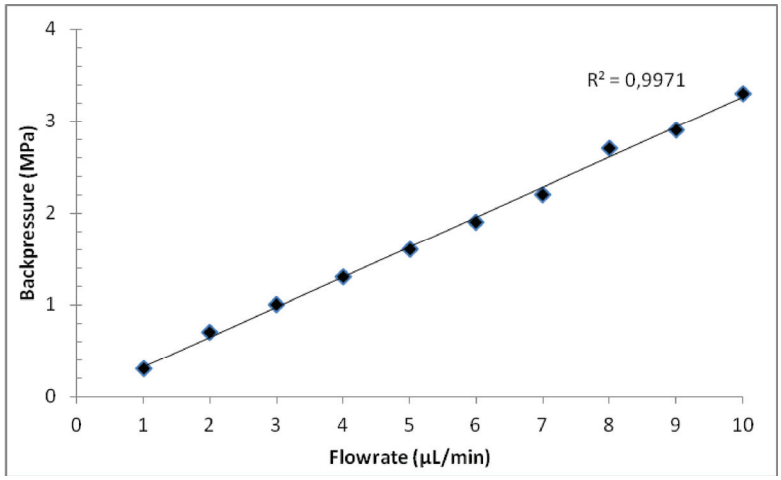

FIGURE 4. Plot of monolith column backpressure vs flowrate

Utilization of Monolith Column for Toluene and Amylbenzene Separation

Furthermore, produced monolith column was then studied to separates toluene and amylbenzene. A solution consisted of 25 ppm of toluene and 75 ppm of amylbenzene was then injected to into the HPLC system using acetonitrile:water (75:25) as mobile phase at UV detection of 214 nm. Fig. 5 shows the toluene and amylbenzene successfully separates sequently at retention time of 84 and 132 min with relative area of 28 and 72%. The sequence of emitted samples due to saturated chain of produced monolith having hydrophobic character. This causes resulting the stronger interaction of amylbenzene with the monolith column than toluene. Later on, toluene with less hydrophobic character was emitted firstly. Wherein separation process took about 200 min which means the total usage of mobile phase is about 1.5 mL.

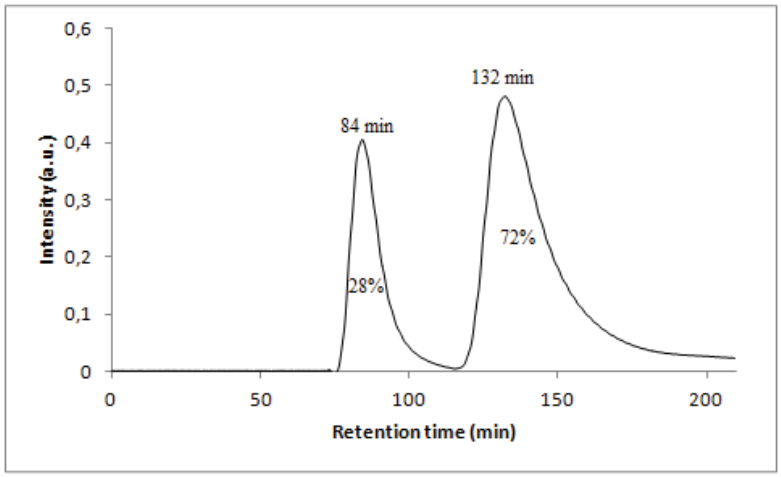

FIGURE 5. Separation of toluene and amylbenzene on monolithic column. Column size: 10 cm long × 1.00 mm i.d.; mobile phase: ACN: (75:25); flowrates: 7.5 µL/min; UV detection at 214 nm

CONCLUSIONS

A monolithic column as HPLC stationary phase has been made from monomer of GMA and TMPTMA inside PEEK tubing. The produced column has capability to separates toluene and amylbenzene with ACN:water (75:25) as mobile phase at 7.5 µL/min. Whereupon the separation process only used about 1.5 mL of mobile phase. Monolithic column as HPLC stationary phase showed promising potential to be developed for separation of natural products.

ACKNOWLEDGMENTS

Our research team would like to thank Direktorat Riset dan Pengabdian kepada Masyarakat (DRPM), Ditjen Risbang, KEMENRISTEKDIKTI RI for helping us in funding this research under the scheme of Penelitian Tim Pasca Sarjana.

REFERENCES

1 A. G. Gilman, T. Rall, S. Alan, and P. Taylor, *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 8th ed. (New York, Pergamon, 1990).
2 C. D. Klaassen, M. . Amdur, L. . Casarett, and J. Doull, *Casarett and Doull's Toxicology: The Basic Science of Poisons*. (New York, McGraw-Hill, 1991).
3 M. S. Melo et al., Biol. Res., **44** (4), 363–368, (2011).
4 G. Zarrini, Z. B. Delgosha, K. M. Moghaddam, and A. R. Shahverdi, Pharm. Biol., **48** (6), 633–636 (2010).
5 D. R. Batish, H. P. Singh, R. K. Kohli, and S. Kaur, For. Ecol. Manag., **256** (12), 2166–2174 (2008).
6 X. Ni et al., BMC Complement. Altern. Med., **12** (1) 253 (2012).
7 J. . Knox and J. . Nelson, N. Engl. J. Med., **275**, 494–1496 (1966).
8 "Toluene," Sigma-Aldrich, Saint Louis Mo, MSDS No. 244511, 2014.
9 "Pentylbenzene," Sigma-Aldrich, Saint Louis Mo, MSDS No. 113174, 2015.
10 R. D. Arrua, M. C. Strumia, and C. I. Alvarez Igarzabal, Materials, **2** (4), 2429–2466 (2009).
11 T. Nema, E. C. Y. Chan, and P. C. Ho, J. Pharm. Biomed. Anal., **87**, 130–141 (2014).
12 Y. Lv, D. Mei, X. Pan, and T. Tan, J. Chromatogr. B, **878** (26), 2461–2464 (2010).
13 C. Yao et al., Talanta, **82** (4), 1332–1337 (2010).
14 P. Zhang, J. Wang, H. Yang, L. Su, Y. Xiong, and F. Ye, RSC Adv, **6** (30), 24835–24842 (2016).
15 Y. Zheng, X. Wang, and Y. Ji, Talanta, **91**, 7–17 (2012).
16 S. Shu, H. Kobayashi, M. Okubo, A. Sabarudin, M. Butsugan, and T. Umemura, J. Chromatogr. A, **1242**, 59–66 (2012).
17 A. Sabarudin, J. Huang, S. Shu, S. Sakagawa, and T. Umemura, Anal. Chim. Acta, **736**, 108–114 (2012).
18 S. Choudhury, L. Fitzhenry, B. White, and D. Connolly, Materials, **9** (3), 212 (2016).