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APPLICATION OF THIOL-MODIFIED SILICA HYBRID MONOLITHS FOR HYDROPHILIC INTERACTION CAPILLARY LIQUID CHROMATOGRAPHY

Firda Furqani1)*, Ilona Bella1), Prima Fitria Hillman1), Lee Wah Lim2)
1)Department of Chemistry, Faculty of Mathematics and Natural Science, Andalas University, Padang, West Sumatera, Indonesia;
2)Department of Chemistry and Biomolecular Science, Faculty of Engineering, Gifu University, Japan;

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**corresponding author:*
firda.furqani@sci.unand.ac.id
Phone: +6282110821882

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ABSTRACT

A silica hybrid monolithic column modified with Thiol-group were successfully prepared via "one-pot" approach; tetramethoxysilane and 3-mercaptopropyltrimethoxysilane were mixed together with the porogens in ice bath before being injected into the fused capillaries (0.032 mm I.D.). The composition of the porogens as well as the reaction time and temperature were optimized. The efficiency of the columns was evaluated by separating some polar biological compounds (i.e. thymine, uracil, adenine, adenosine, and cytidine) under hydrophilic interaction chromatography (HILIC) mode. Uracil, adenosine and cytidine were baseline-separated when the concentration of acetonitrile, which was used as the eluent, was more than 90%. It is also found that by increasing the acetonitrile concentration, the retention time of all analytes was increased, proving that typical HILIC separation mechanism was involved.

INTRODUCTION

Monolithic columns are typically divided into three categories: organic polymer-based, inorganic silica-based, and organic–inorganic hybrid types. Organic polymer-based monoliths offer high biocompatibility and broad pH stability but suffer from limited mechanical strength due to structural swelling and shrinkage (Ganewatta & El Rassi, 2020; Hefnawy et al., 2023; Si et al., 2022). In contrast, silica-based monoliths exhibit superior solvent resistance and rigidity, though their fabrication is time-consuming and difficult to control (Eeltink et al., 2021; Sharma et al., 2017; Staniak et al., 2020). Therefore, hybrid monolithic columns, which combine the advantages of both materials-simple preparation, reduced solvent swelling, and good mechanical stability, have received increasing attention (Ma et al., 2020; Zajickova, 2023). Their performance depends strongly on factors, i.e., the silica precursors ratio, monomer content, and reaction conditions.

Hybrid monoliths have been prepared via several routes, including free-radical (Ma et al., 2017; Zhang et al., 2015), photo-initiated (Ma et al., 2018), and ring-opening polymerization (Lin et al., 2015; Wang et al., 2016), as well as thiol-ene click chemistry (Sun et al., 2020; Wang et al., 2015). Despite its potential, the one-pot thiol–ene click approach remains scarcely explored. Introduced by Sharpless et al. (2001), click chemistry comprises rapid, selective, and high-yield reactions through heteroatom linkages (C–X–C). The thiol–ene variant is particularly attractive due to its simplicity, efficiency, chemoselectivity, and nearly complete conversion under mild conditions (Liu et al., 2017; Wang et al., 2017). Click reactions used in monolith fabrication primarily include copper-catalyzed azide–alkyne cycloaddition, thiol-ene, and thiol-methacrylate reactions (Schmidt & Bottoli, 2019).

Previous studies have successfully employed thiol-ene reactions to introduce various functionalities onto hybrid monoliths, mainly for reversed-phase, affinity, or mixed-mode separations. In most reported cases, thiol-ene reactions were conducted as a post-polymerization modification rather than integrated directly into a one-pot fabrication process. Liu *et al.* synthesized octadecyl-functionalized hybrid silica *via* vinyl surface modification with 1-octadecanethiol, achieving effective separation of nonpolar compounds and proteins. Lin *et al.* combined free radical polymerization and click chemistry to prepare a poly (3'-azido 3'deoxythymidine-co-propargyl methacrylate-co-pentaerythritol triacrylate) monolith, enabling high-efficiency separation of alkylbenzene, amides, anilines, benzoic acid, and proteins (Lin et al., 2015).

To date, one-pot thiol-ene-based hybrid silica monoliths that directly function as HILIC stationary phases remain scarcely explored. In this study, a hybrid monolithic column was synthesized via a one-pot thiol-ene click reaction. The thiol functionalities introduced onto the monolith surface serve as hydrophilic interaction liquid chromatography (HILIC) sites for the separation of polar compounds. HILIC-type monoliths effectively separate some polar compounds and can be applied for anion and cation separations through ion-exchange-like mechanisms (Y. Liu et al., 2023; Sari et al., 2018)

MATERIALS AND METHODS

Materials

Materials used in this study were Tetramethoxysilane (TMOS), 3-mercaptopropyltrimethoxysilane (MPTMS), Poly(ethylene glycol) (PEG, MW 10000), Urea, and acetic acid.

Methods

Preparation of the monolithic column was carried out by mixing TMOS, the primary silica precursor, with MPTMS using the sol–gel method. The silica precursors were combined with porogens (PEG with a molecular weight of 10,000, urea, and acetic acid) via the sol–gel process for 1 hour at 0 °C. The mixture was then introduced into the pretreated capillary, both ends sealed with PTFE tubing, and polymerized at 40-70 °C for 8-24 h. The resulting solution was then manually introduced into a pre-treated fused-silica capillary, and both ends of the capillary were sealed with Teflon caps. The column was then incubated at various temperatures. The morphology of the resulting capillary monolithic column was examined using a scanning electron microscope (SEM). Chromatographic performance was evaluated using a CLC system with a 0.2 µL injection volume, a UV-2075 UV detector at 254 nm, and a mobile-phase flow rate of 3 µL/min.

RESULTS AND DISCUSSION

Preparation of thiol-functionalized hybrid monolithic columns

A hybrid monolithic column was synthesized via a one-pot process (Fig. 1). The amounts of silica precursors and porogenic agents were kept constant in all experiments: TMOS (180 mg), MPTMS (60 mg), PEG (MW 10000; 54 mg), urea (45 mg), and acetic acid (0.5 mg). The effects of reaction temperature and reaction time on the formation of the Si–MPTMS hybrid monolith were then examined, and the optimized conditions were subsequently applied to the preparation of the thiol–ene click reaction-based hybrid monolithic column. TMOS and MPTMS were employed at a mass ratio of 3:1 (180 mg:60 mg). This ratio was selected based on previous studies on hybrid silica monoliths, which reported that a higher proportion of tetraalkoxysilane is required to maintain a mechanically stable silica network, while a moderate amount of functional organosilane ensures sufficient surface functionality without disrupting sol–gel condensation and pore formation (Wang et al., 2017).

During the sol-gel process, TMOS and MPTMS undergo acid-catalyzed hydrolysis, in which the methoxy groups are converted into silanol (Si-OH) species. These silanol groups subsequently undergo condensation reactions to form Si-O-Si linkages, leading to the development of a hybrid silica network. The co-hydrolysis of TMOS and MPTMS allows the thiol-functionalized organosilane to be uniformly incorporated into the monolithic matrix, ensuring both structural integrity and the presence of polar surface functionalities (Baskaran et al., 2022).

The monoliths formed at 40 ºC and 50 ºC exhibited homogeneous porous structures, whereas higher temperatures produced denser, non-porous morphologies. The permeability decreased from 4.20 x 10^{-13} m^2 to 1.49 x 10^{-13} m^2 with increasing temperature, likely due to enhanced porogen solubility and delayed phase separation, resulting in a more compact structure.

The influence of reaction time was also examined using samples prepared for 8, 12, and 24 h (columns E-G). Permeability varied only slightly among these samples, with the highest value 3.78 x 10^{-13} m^2 observed after 24 h of reaction. To reduce synthesis time, a 12 h reaction period was selected for subsequent preparations.

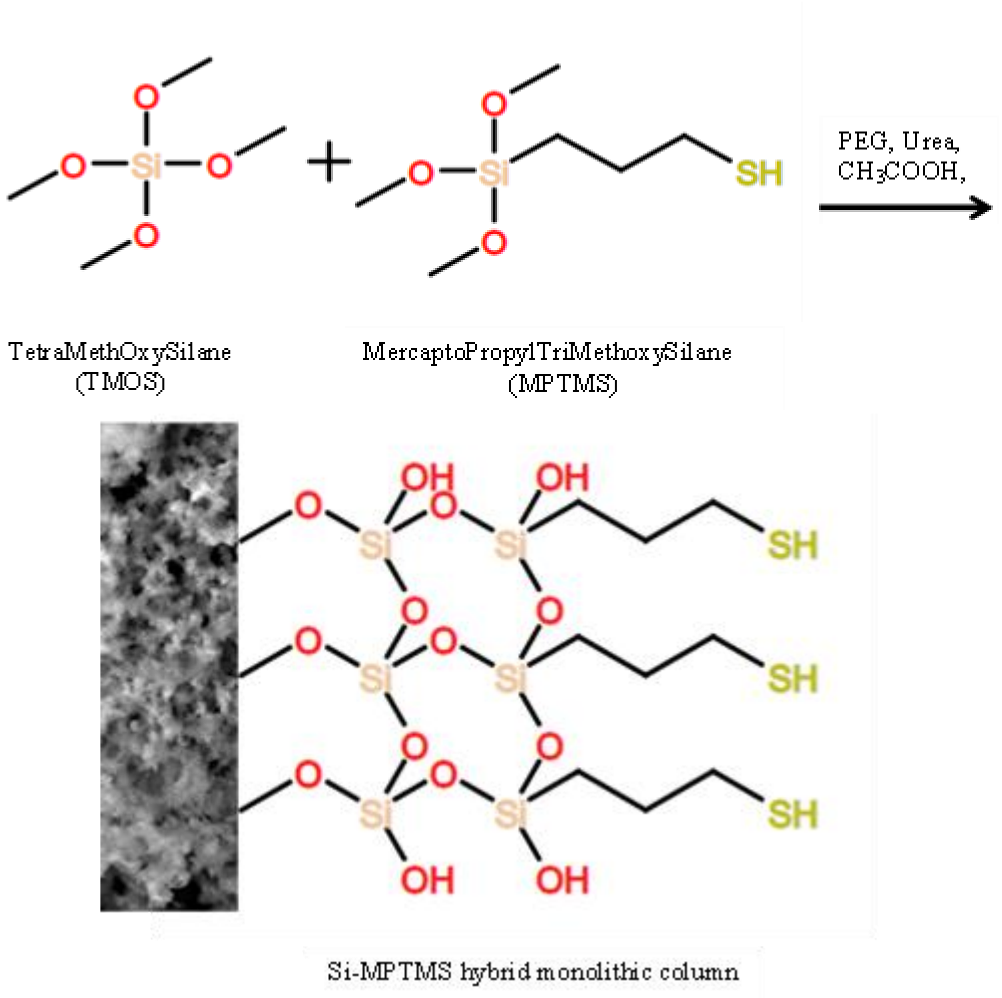

Figure 1 Expected reaction for preparation of the Si-MPTMS hybrid monolithic columns

Table 1. Preparation conditions for Si–MPTMS hybrid monolithic column

Column	t (h)	T (°C)	Monolith Condition	Permeability (*k*) (10^{-13} m^2)
A	24	40	agglomeration	4.00
B	24	50	homogeneous	4.20
C	24	60	packed, high pressure	1.49
D	24	70	packed, high pressure	1.50
E	8	50	homogeneous	3.05
F	12	50	homogeneous	3.60
G	24	50	homogeneous	3.78

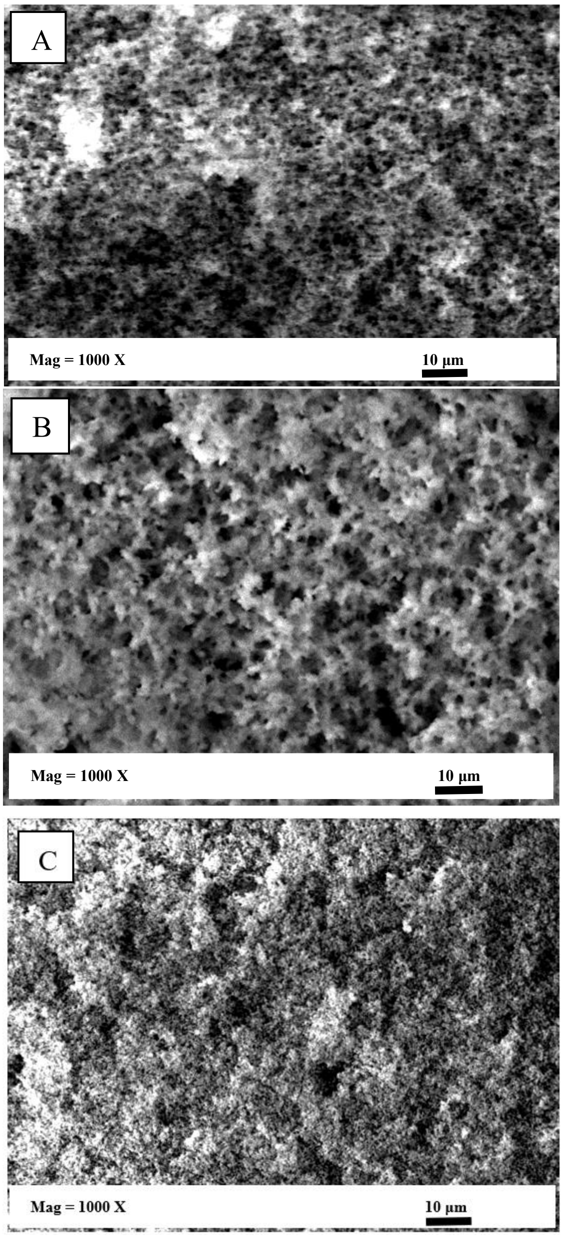

Figure 2 SEM micrographs of Si–MPTMS hybrid monolithic columns synthesized at various reaction temperatures. A: 40ºC, B: 50ºC, C: 60ºC.

Chromatographic performance of the hybrid monolithic column for polar compounds

The chromatographic performance of the Si-MPTMS hybrid monolithic column was evaluated using several polar analytes, including thymine, uracil, adenine, adenosine, uridine, and cytidine. As shown in Fig. 3, the separation of these compounds on monoliths prepared under different reaction times using 90% acetonitrile as the mobile phase. Five analytes were successfully resolved; however, thymine and uracil co-eluted under the applied conditions, indicating similar polarity and retention characteristics. The separation behavior reflects the hydrophilic interaction mechanism between the polar analytes and the thiol-silica surface of the monolith. Retention was dominated by hydrophilic interactions by partitioning between the water-enriched stationary and organic-rich mobile phases, consistent with a HILIC-type retention mode. The incomplete resolution of thymine and uracil suggests that further optimization of eluent composition or gradient conditions, such as reducing acetonitrile content or adjusting buffer strength, could enhance selectivity among structurally similar analytes. Compared with other HILIC monolithic stationary phases, such as diol, amide, and zwitterionic functionalized monoliths, the Si-MPTMS hybrid monolith developed in this study exhibits a simpler surface chemistry with predominantly neutral polar functionalities. Diol and amide-based HILIC phases rely mainly on hydrogen bonding, while zwitterionic phases introduce additional electrostatic interactions that may enhance selectivity but often increase sensitivity to buffer composition. In contrast, the thiol-functionalized surface provides stable HILIC-type retention dominated by partitioning effects, while preserving the advantage of straightforward one-pot fabrication and the potential for further post-functionalization via thiol-ene click chemistry.

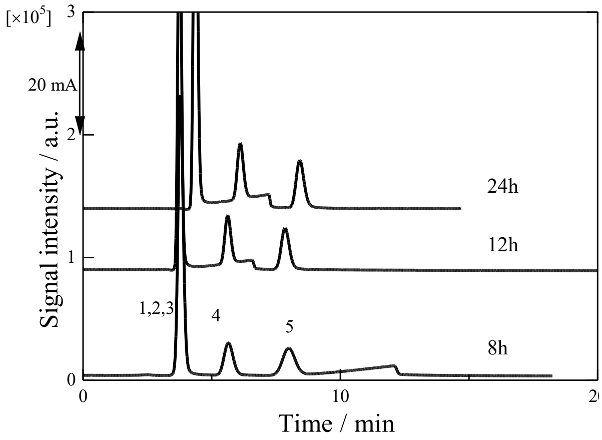

Figure 3 Separation of polar analytes using Si-MPTMS hybrid monolithic columns; analytes: 1:thymine, 2:uracil, 3:adenine, 4:adenosine, 5:citydine

Effect of mobile phase of separation on polar compounds

Fig. 4 illustrates the effect of acetonitrile concentration in the mobile phase on the retention of polar analytes. The acetonitrile content was varied from 90% to 98%. As the proportion of acetonitrile increased, the retention times of all analytes also increased, confirming a typical HILIC-type retention mechanism. This behavior can be attributed to the enhanced formation of a water-enriched layer on the monolith's hydrophilic surface at higher organic solvent concentrations, which strengthens partitioning interactions between the analytes and stationary phase. At lower acetonitrile concentrations, the thinner water layer reduces hydrophilic interactions, resulting in shorter retention times. These results confirm that the hybrid monolithic column provides stable HILIC performance and can effectively separate a wide range of polar compounds by adjusting the acetonitrile-to-water ratio in the mobile phase.

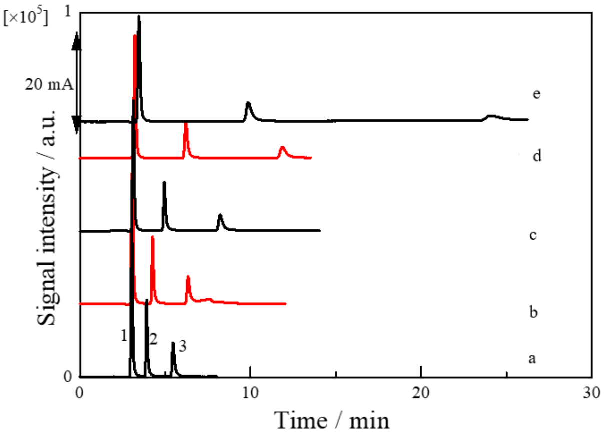

Figure 4 Separation of polar analytes using Si-MPTMS; Mobile phase: Acetonitrile a. 90%, b. 92%, c. 94%, d. 96%, e. 98%; analytes: 1:uracil, 2:adenosine, 3:citydine

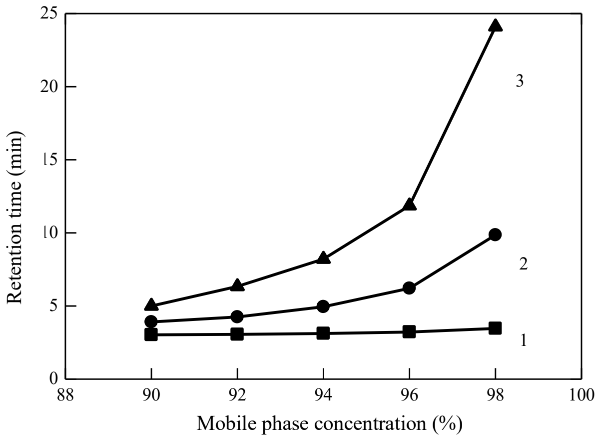

Figure 5 Time Plot of mobile phase concentration versus retention time of analyte; analytes: 1:uracil, 2:adenosine, 3:citydine

CONCLUSIONS

A Si–MPTMS hybrid monolithic column was successfully synthesized via a one-pot approach and demonstrated effective separation of several polar compounds, including thymine, uracil, adenine, adenosine, and cytidine, using 90% acetonitrile as the mobile phase. Increasing the acetonitrile concentration resulted in prolonged retention times for all analytes, confirming a characteristic HILIC-type retention mechanism. These findings indicate that the developed Si–MPTMS hybrid monolith provides stable and reproducible performance for the separation of polar compounds. The optimized preparation conditions established in this study will serve as a foundation for future work on the fabrication of hybrid monolithic columns functionalized with thiol groups for thiol-ene click reactions with tailored monomers, aiming to enhance selectivity and expand application versatility.

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