Poly(Styrene-Divinyl Benzene)-Based Monolithic Column For High Performance Liquid Chromatography

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Abstract

Poly(styrene-divinyl benzene) (PS-DVB) monoliths were synthesized by in-situ polymerization in a 2.1 mm × 150 mm high performance liquid chromatography, (HPLC) column. The physical properties of the monolith were studied by FTIR and SEM. IR spectra of the monolith confirmed the formation of PS-DVB whereas SEM analysis shows that the PS-DVB monolith gave the cauliflower structure. The performance of the synthesized column was evaluated by HPLC and compared with commercial PS-DVB column. The separations were carried out using 4 test compounds; benzaldehyde, nitrobenzene, phenol and toluene. Although the monolith column was less efficient than commercial packed PS-DVB column, the monolith gives faster analysis time.

Keywords: Poly(styrene-divinylbenzene); Monolithic Column

1. Introduction

Although many chromatographers feel high performance liquid chromatography (HPLC) is a mature technique, chromatography research continues to make progress. One approach to develop high-throughput HPLC method is using monolithic column. Recently, preparations of organic polymer-based monolithic stationary phases in capillary columns have attracted increasing attention in liquid chromatography. The key advantages provided by polymer-based monolithic columns include the easy preparation, high performance, modifiable properties such as porosity, surface area and functionality, chemical stability over the pH range of 1 – 14 and absence of frits to retain the packed bed compared to a conventional columns packed with particles for the separation of biopolymers [1].

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Several previous study reports on the development of monolithic column with an internal diameter in a range of micrometer. This research will focus in the development and performances study of Poly(styrene-divinylbenzene) (PS-DVB) monolithic column for 2.1 mm i.d. PS-DVB copolymers are the most widely polymeric material used in chromatographic analysis. Resins based PS-DVB are stable with eluents from pH 1-13 and overcome many of the limitation of bonded silicas especially the pH stability of the silica under acidic or basic condition and the presence of residual silanol groups that can cause peak broadening or tailing by interaction with polar compound [2].

The group of Premstaller showed excellent applications of this polymer-based material in 2000. The porogen in this case was a mixture of tetrahydrofuran and decanol. This mixture would produce mesopores for the separation and macropores for the fast analysis of compounds [3]. In their paper, the gradient elution ion pairing reversed-phase analysis of

single and double stranded nucleic acids was achieved with effiencies of 190 000 plates/m. Furthermore, the capillary columns were hyphenated to a mass spectrometer via electrospray ionization, enabling the mass recognition of the nucleic acids.

2. Method

2.1. Reagents

Styrene (99%, stabilized with 0.005% 4-tent-butylcatecol) was from Fluka Chemika (Switzerland). The styrene monomer was pre washed consecutively with 10% sodium hydroxide (50 ml × 3) and distilled water (50 ml × 3) prior to use. Technical-grade divinylbenzene (DVB) used as the crosslinker was laboratory grade (70-85%) stabilized with 0.2% 4-tert-butylcatecol obtained from Merck (Schuchardt, Germany) and Fluka Chemika. The DVB was purified with 10% sodium hydroxide (50 ml × 3) and distilled water (50 ml × 3) before use. Azobis(isobutyronitrile) (AIBN) used as initiator without any purification. Tetrahydrofuran, THF were purchased from Fisher Scientific (USA) and ethanol was obtained from Fluka Chemica. Double-distilled deionized water of at least 18 MΩ was purified by Nano ultra pure water system (Barnstead, USA). HPLC grade acetonitrile (100%) was obtained from J. T. Baker (USA). Absolute methanol (100%) was from MERCK (Germany). The test substances, nitrobenzene was from Sigma-Aldrich (Germany), phenol from Hopkin & Williams (England), toluene from MERCK (Germany) and benzaldehyde from Riedel-de Haen (Germany).

2.2. Procedure

2.2.1 Development of PS-DVB monolith

The polymer mixture consisted of 2 mL THF, 5 mL styrene, 5 mL DVB, 13 mL ethanol and 10 mg/mL of initiator (0.25 mg AIBN). The mixture was then stirred using a magnetic bar until all chemicals dissolved. A length of an empty column was then filled with the polymer mixture using a syringe. Septa were then placed at both ends of the column to seal the column and left to polymerize for 24 h at 70°C.

2.2.2 *Chemical Characterization*

- **Fourier Transform Infrared (FTIR)**
  The characterization of PS-DVB by far region IR was carried out to detect the functional groups contains in the sample and structure confirmation. FTIR used was from Shidmadzu FTIR 8300.
- **Scanning Electron Microscopy (SEM)**
  Samples were placed on an aluminium made stub which was covered with double-sided tape. The tape was used to eliminate any possible discharge of samples from the surface when the scanning was done. After the sample was well spread on the surface of the tape, the sample was coated with gold (aurum). This coating step was carried out to ensure that the sample was able to undergo electron bombardment without any discharge effect. Scanning was done using a Philips XL 40 and the bombardment using electron gun with tungsten filament under 30kV resolution to get the scanning image.

2.2.3 Reversed Phase High Performance Liquid Chromatography (RP-HPLC) Instrumentation.

The HPLC systems consisted of a conventional HPLC system coupled with a column oven of a Shimadzu GC-8A Gas Chromatography (Shimadzu Kyoto, Japan). HPLC separations were carried out using Waters 515 HPLC pump (Mildford, USA) for mobile phase delivery. Samples were injected into the system using a 25 µL loop. Analyte peaks were detected using a Shimadzu SPD-6A UV detector (Kyoto, Japan) and were recorded on a Waters 746 Data Module integrator (Mildford, USA).

A 30 cm × 0.5 mm i.d. length of stainless-steel tubing was placed in the oven between the injection valve and the column as pre-heating coil. The column and the preheating coils were placed together in the oven. The columns type and dimension used in this research are as shown in Table 1.

Table 1: Columns type and dimension used in the study

Product name and description	Diameter (mm)	Length (mm)
PLRP-S 100 Å (5μm PS-DVB) (commercial)	2.1	150
Monolithic PS-DVB	2.1	150

Mobile phase used in this research was degassed using a vacuum-ultrasonic degassing procedure. Mobile phase was degassed in the ultrasonic bath (NEY 300 Ultrasonic, USA) to release the gas from reservoir. The instrument set-up for RP-HPLC is shown in Figure 1.

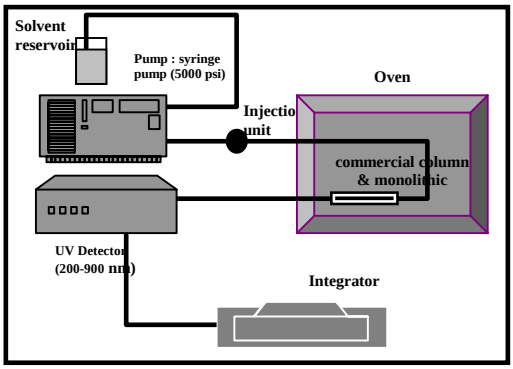

Figure 1: Diagrams of high temperature high performance liquid chromatography system.

2.2.4 System Conditioning Procedure

Mobile phase used was prepared by mixing deionized water from the laboratory with HPLC grade organic modifier acetonitrile. The mobile phase were degassed by using a vacuum ultrasonic method. Each time the system was turned on, the HPLC system was eluted at a flow rate of 0.5 mL/min and flushed overnight at a flow rate of 0.1 – 0.2 mL/min to equilibrated the column. The GC oven was used to heat up the column, at least 30 minutes was used to stabilize the column temperature and equilibrium of the whole system. The detector was warmed up for at least 15 minutes after stabilizing the system.

2.2.5 Performance Study of the Column

Four compounds were used as the test compounds consisted of phenol, benzaldehyde, nitrobenzene and toluene. Mixture of the analytes was prepared to produce mixture solutions of 100 μg/mL for each analytes except toluene at 2000 μg/mL. The mobile phase consisted of a mixture of acetonitrile-water (50:50 v/v) eluted at a flow-rate of 0.2 mL/min. Approximately 1 μL of sample mixture was injected into the HPLC system and was repeated three times each under identical chromatographic conditions. The temperature was maintained at approximately 50°C. The detection wavelength was set at 254 nm and sodium nitrate was use to determine the void volume or t_o. Performance of the column was examined by analyzing the retention factor, *k'*; separation factor, α ; theoretical plate number, N ; plate height, H and resolution, Rs.

3. Results and Discussions

The experiment was carried out via *in-situ* polymerization and the monolith was prepared in 2.1 mm i.d. and 150 mm length. The physical properties of the monolith were investigated using FTIR and SEM [4].

The IR spectra for both synthesized PS-DVB monolith and commercial PS-DVB are present in Figure 2. It was observed that both spectra gave the similar absorptions patterns as displayed in Table 2. Hence, it could be concluded that the structures of PS-DVB monoliths are similar to the commercial PS-DVB and the monoliths have been successfully synthesized via *in-situ* polymerization.

Table 3.2: Absorption peaks and frequencies for PS-DVB monolith and commercial PS-DVB

Absorption peaks	ν_{max} (cm^{-1}) Commercial PS-DVB	ν_{max} (cm^{-1}) PS-DVB monolith
C-H sp^3 (stretching)	3002.0	2850.6
CH_2	3025.1	2922.0
C-H aromatic	3058.9	3026.1
C=C aromatic	1600.8 and 1450.4	1600.8 and 1450.4
monosubstitude C=C	758.0 and 699.1	758.0 and 698.2

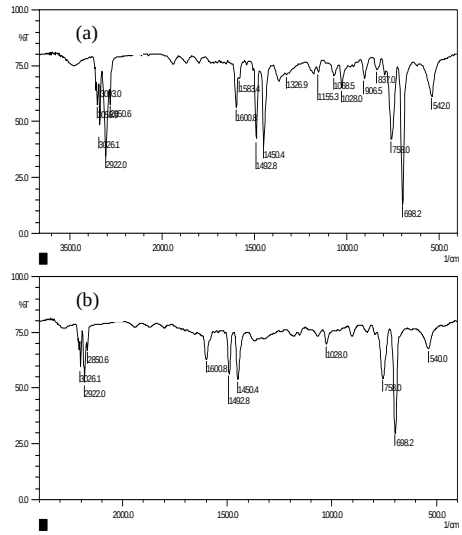

Figure 2: IR spectra of PS-DVB monoliths; (a) commercial PS-DVB; (b) PS-DVB monolith in 2.1 mm i.d column.

A general view from the SEM (Figure 3a) shows that the particles in the commercial PS-DVB resemble spherical beads. Otherwise, the monolith morphology consists of globules, which are cross-linked to form a continuous network like cauliflower structure as shown in Figure 3b. This phenomenon appears due to specific morphology in monolithic column. The monolith structure is composed of benzene rings linked by sp^3 carbons. This conformation gives the backbone a hydrophobic character ideal for reversed-phase chromatography [5].

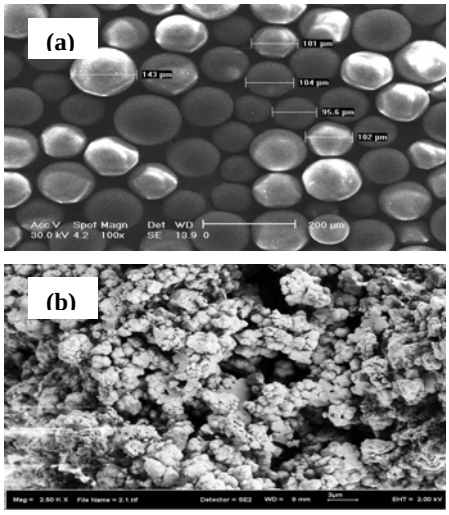

Figure 3: SEM of (a) commercial PS-DVB with magnification 100×; (b) Synthesis PS-DVB monolith with magnification 2500×

All test compounds were successfully eluted within 15 minutes with the monolith. For individual run (Figure 4a, b and c), it shows that benzaldehyde was first eluted, followed with nitrobenzene and toluene. However, for the mixture only two peaks were successfully separated, benzaldehyde and toluene (Figure 4d). This possibly occurred because each peaks in the mixture quite broader, so combining them to form a mixture will enhance the overlapping.

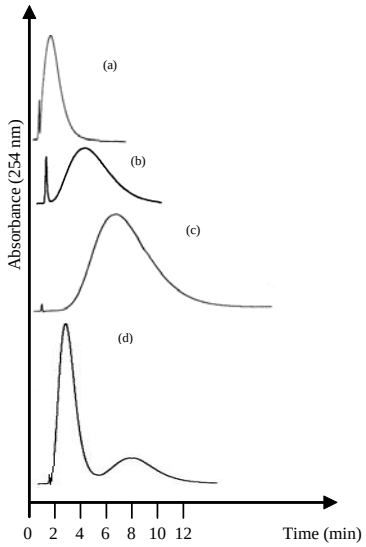

Figure 4: Separation of individual test analyte on PS-DVB monolithic column (150 × 2.1 mm i.d). Chromatographic condition: mobile phase: acetonitrile-water 50:50 (v/v); flow rate: 0.5 mL/min; detection: UV absorbance at 254 nm. Peaks (a) benzaldehyde; (b) nitrobenzene; (c) toluene; (d) mixture: benzaldehyde and toluene

Figure 5 shows the comparison of performances between commercial PS-DVB column and the monolith. It shows that all test compounds were successfully separated using commercial column and an elution order start from phenol, benzaldehyde, nitrobenzene and finally toluene. On the other hand, as describes earlier only two peaks separated by the monolith.

Referring to the toluene peak, it shows the commercial PS-DVB column give *k'* value of 34.54; R_S, 9.25; α, 2.00; *N*, 5 863 and *H* value of 25.5842×10^{-4}. For the monolithic column, the value of *k'* is 8.65; R_S, 3.04; α, 5.68, *N*, 985 and *H* 1.52284×10^{-4}. Under approximately identical chromatographic condition, results show that commercial PS-DVB column is

more efficient than monolithic column. However, the most important finding from this experiment was that the monolith actually performed a much quicker analysis. It was observed that the analysis was more sensitive when the monolithic column was used. The analytes interact less with the PS-DVB phase and as a consequence they can elute at an earlier stage [6].

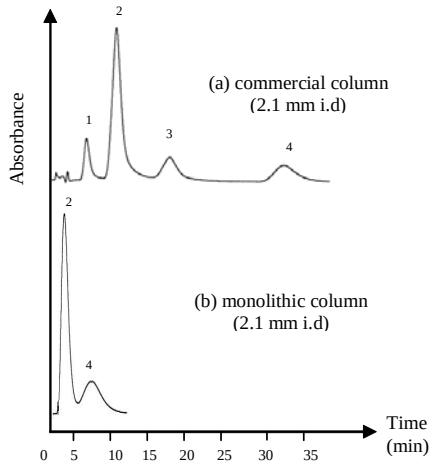

Figure 5: Separation of test compound on (a) commercial PS-DVB column (PLRP-S column; 150 × 2.1 mm i.d). (b) PS-DVB monolithic column (150 × 2.1 mm i.d). Chromatographic condition: mobile phase: acetonitrile-water 50:50 (v/v); flow rate: 0.2 mL/min; detection: UV absorbance at 254 nm. Peaks: 1 – phenol; 2 – benzaldehyde; 3 – nitrobenzene; 4 – toluene.

4. Conclusions

Based on physical analysis, PS-DVB monolith in 2.1 mm i.d column was successfully synthesized via *in-situ* polymerization. Commercial packed PS-DVB column is more efficient for separation analysis compared with the PS-DVB monolithic column. However, it shows that the monolith could reduce the analysis time.

Further study could be conduct to develop PS-DVB monolith with better separation efficiency. Focus will give in method of synthesizing the monolith to increase its sensitivity. Several parameters in synthesizing the monolith will be measured such as temperature, types of solvent and reaction time.

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