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Preparation of a novel porous poly (trimethylol propane triacrylate-co-ethylene dimethacrylate) monolithic column for highly efficient HPLC separations of small molecules

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ABSTRACT

A novel poly (trimethylol propane triacrylate-co-ethylene dimethacrylate) [poly (TMPTA-co-EDMA)] monolith was prepared by in situ free-radical polymerization in a 50 mm × 4.6 mm i.d. stainless steel column and was investigated for high performance liquid chromatography (HPLC). The porous structure of monolith was optimized by changing the conditions of polymerization. The chemical group of the monolithic column was confirmed by a Fourier transform infrared spectroscopy (FT-IR) method and the morphology of column structure was characterized by scanning electron microscopy (SEM). The mechanical strength and permeability were also studied. Finally, a series of low-molecular-weight organic compounds were utilized to evaluate the retention behaviors of the monolithic column. The result demonstrated that the prepared column exhibited an RP-chromatographic behavior and good separation performance. The method reproducibility was obtained by evaluating the run-to-run and column-to-column with relative standard deviations (RSDs) less than 0.7% ($n=6$) and 2.9% ($n=6$), respectively, which indicated that prepared monolithic columns had good reproducibility and stability.

1. Introduction

In recent years, the monolith as separation media for high performance liquid chromatography (HPLC) has undergone a rapid development in the field of sample analysis because of its excellent performance [1,2], such as cost-effective approach, fast mass transport, excellent permeability and versatile surface modification compared to conventional columns packed with particles [3–5]. Monolithic column are divided into three groups: organic polymer-based, silica-based and organic-silica hybrid monolithic columns. Silica-based monoliths can be applied for high-throughput analysis and rapid separation, but the preparation process is complicated. The silica-based hybrid monoliths are famous for their better separation efficiency, but the drawback is that the Si–O–C linkage cannot be hydrolyzed fully. In addition, the synthetic process is difficult to control and the preparation is time-consuming [6]. Organic polymer-based monolithic column including polyacrylates, polymethacrylates [7], polyacrylamids [8] and polystyrenes [9] show excellent biocompatibility, good stability of pH changes and easy surface modification. Lots of applications have been already put into effect in recent years [10–12], although organic polymer-based monolithic columns still have some disadvantages which need to be improved.

Trimethylol propane triacrylate (TMPTA) belongs to polyolacrylate that can be used as important multifunctional monomers. TMPTA could be candidate for polymer network, since it has three vinyl bonds at the end to be formed a dense network structure [13–17]. So far, in the field of monolithic stationary phase for HPLC, no significant attempts have been made with TMPTA.

In this work, a novel HPLC monolithic column was synthesized via in situ free-radical polymerization using TMPTA and ethylene dimethacrylate (EDMA) as monomer and cross linker, respectively. The influence factors on the preparation of the monoliths have been studied. Furthermore, the newly monolith was used to separate a series of small molecules.

2. Experimental

2.1. Materials

Trimethylol propane triacrylate (TMPTA) was supplied by Tianjin Tianjiao Chemical Co., Ltd. (Tianjin, China). Ethylene dimethacrylate (EDMA) was purchased from Acros (New Jersey, USA). 2,2'-azobisisobutyronitrile (AIBN) was produced by Shanghai Chemical Plant (Shanghai, China) and refined before use. Poly (ethylene glycol) (PEG, Mn=200) and methanol were obtained from Tianjin Kemiou Com (Tianjin, China). The aromatic compounds were provided by the National Institute for the Control of Pharmaceutical and Biological

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Products of China (Beijing, China). All other chemicals were of analytical grade or better. Triplex distilled water was used for all experiments. All media were filtered through a 0.45 μm membrane before use.

2.2. Instruments

All chromatographic experiments were performed on a 1100 system from Agilent Technologies (Shanghai, China). Agilent liquid chromatography system software was used and operated under Windows XP for data acquisition. The FT-IR spectra were recorded on an FTIR-8400S IR apparatus in the region of 400–4000 cm^{-1} (Shimadzu, Kyoto, Japan). Scanning electron microscopy (SEM) of the monolithic columns was carried out on a Hitachi S-4300 SEM instrument (Hitachi High Technologies, Tokyo, Japan).

2.3. Preparation of the poly (TMPTA-co-EDMA) monolithic column

The polymerization mixture for the monolithic columns was prepared as follows: 0.3 mL TMPTA, 0.3 mL EDMA, and 0.005 g AIBN were dissolved in a mixture of 0.5 g PEG and 1.4 mL methanol, which had been injected into a dry ampule. The mixture solution was sonicated for 30 min after being shook for 2 min and then bubbled with nitrogen for another 5 min to reinforce dissolve and remove gases. Then, pour the reaction solutions into a 50 mm × 4.6 mm i.d. stainless steel column that was sealed at both ends. The stainless steel was heated up to 60 °C in a water bath for 24 h. After that, the seals were removed and provided with end fittings. In order to remove all of unreacted monomers and soluble compounds, the monolith was washed by methanol online for 1 h at a flow rate of 1 mL/min. The scheme of polymerization was shown in Fig. 1.

2.4. Characterization method

A Fourier transform infrared spectroscopy (FT-IR) method was used to confirm the chemical group of the monolith. Before the measurement, a piece of monolith was grinded into powder, and then put it in a plate for drying 48 h in vacuum at 70 °C. 1 mg of the dried sample and 200 mg of KBr powder were weighed. The mixture was grounded in an agate mortar to pestle uniformly. After that, it was pressed to form a pellet, loaded it on the specimen holder, and then a transmission spectrum with a sharp peak was obtained. Morphology of the monolithic materials was studied by scanning electron microscopy (SEM). Prior to the SEM, the monolithic column should be rinsed in HPLC with methanol until a stable baseline was observed to ensure any soluble compounds were removed. Subsequently, the monolith was cut into small pieces after pushing out from stainless steel column and then dried in vacuum at 50 °C for 24 h. Then, using a small fragment of monolith sputtered with gold to carry out SEM.

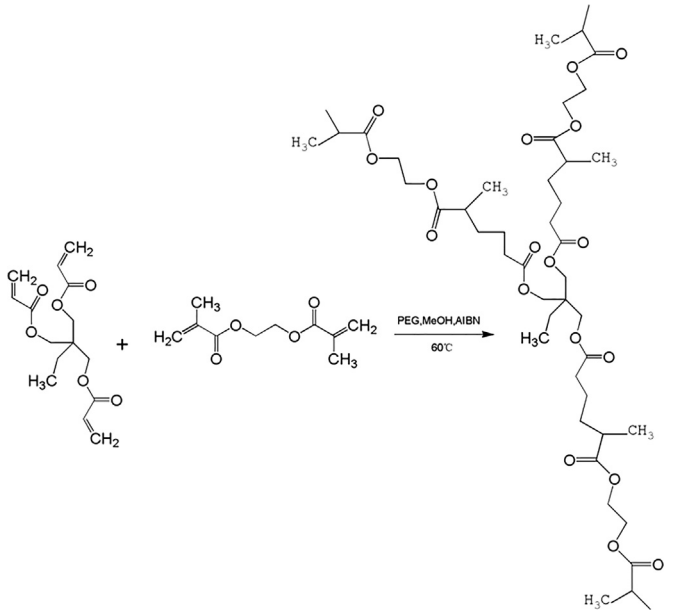

Fig. 1. Synthesis scheme of the poly (TMPTA-co-EDMA) monolithic column.

2.5. Preparation of solutions

All the solutions, including *p*-xylene, 1H-benzotriazole, phenol, α-naphthol, biphenyl, phenanthrene, and 1, 2-pheneylenediamine, 1-naphthylamine, *p*-methoxy azobenzene were dissolved in the methanol (0.1 mg/mL), which were sealed and stored at 4 °C before separated for HPLC.

2.6. HPLC conditions

The HPLC system equipped with a quaternary pump, a UV detector and an autosampler with variable injection capacity from 0.1 to 100 μL. A monolithic column was prepared with a total length of 50 mm × 4.6 mm i.d. stainless steel column. The mobile phase was the mixture of water and methanol, the UV wavelength was set at 254 nm. The room temperature was 25 °C. The sample injection volume of the autosampler was 1.0 μL.

2.7. Calculation

The ability of liquid passing the material is expressed by permeability, which reflects through-pore size and external porosity. The permeability (K) of monolithic columns was calculated by the following equation:

$$K = \frac{F \times \eta \times L}{\Delta P \times \pi \times r^2} \quad (1)$$

where F is volume flow rate of the mobile phase, η is phase dynamic viscosity of the mobile phase, L is the column length, ΔP is the column back pressure and r is the inner radius of the column [18]. In this work, methanol was used as mobile phase and its corresponding value of dynamic viscosity was 0.580×10^{-3} kg/(ms) at 25 °C [19].

The retention factor (k) of each aromatic compound on poly (TMPTA-co-EDMA) monoliths at different mobile phase for HPLC separation was determined by the equation, $k=(t_R-t_0)/t_0$, where k, t_0, t_R, stand for the retention factor, the retention time of aromatic compounds, and the retention time of void marker, respectively. The thiourea was selected as the void time marker in this experiment.

Theoretical plate number (N), one of the parameters of the chromatographic column efficiency, is a quantitative representation,

Table 1
Compositions of the mixtures used for preparation of monolithic columns and their permeability.

Column	EDMA (mL)	TMPTA (mL)	MeOH (mL)	PEG (g)	AIBN (g)	Back pressure[a] (bar)	Permeability K ($\times 10^{-14}$ m^2)
A	0.3	0.3	1.4	0.5	0.005	6	1.3388
B	0.3	0.3	1.0	0.9	0.005	7	1.1469
C	0.2	0.3	1.4	0.5	0.005	> 11	No date
D	0.4	0.3	1.4	0.5	0.005	5	1.6065
E	0.2	0.4	1.4	0.5	0.005	> 16	No date
F	0.4	0.2	1.4	0.5	0.005	4	2.0081

[a] Back pressure is obtained with methanol as the mobile phase at 1 mL/min, and the length of the stainless steel column was kept at 5 cm.