Journal of Chromatography A, 1324 (2014) 128–134

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

High-performance liquid chromatography separation of small molecules on a porous poly (trimethylol propane triacrylate-co-N-isopropylacrylamide-co-ethylene dimethacrylate) monolithic column

Haiyan Liu*, Xiaomei Bai, Dan Wei, Gengliang Yang*

College of Pharmacy, Hebei University, Hebei Province Key Laboratory of Pharmaceutical Quality Control, Baoding 071002, China

ARTICLE INFO

Article history:
Received 1 August 2013
Received in revised form 31 October 2013
Accepted 13 November 2013
Available online 19 November 2013

Keywords:
High performance liquid chromatography
Monolithic column
N-isopropylacrylamide
Small molecule compounds

ABSTRACT

A porous monolith was prepared by in situ free-radical polymerization using N-isopropylacrylamide (NIPAAm) and trimethylol propane triacrylate (TMPTA) as functional monomers, ethylene dimethacrylate (EDMA) as crosslinking agent. The chemical group of the monolith was assayed by a Fourier transform infrared spectroscopy (FT-IR) method and the morphology of optimized monolithic column was characterized by scanning electron microscopy (SEM). The mechanical strength and permeability have been studied in detail as well. The run-to-run and column-to-column reproducibility of the retention times were less than 0.9% and 3.0%, respectively. Furthermore, the influence of temperature and mobile phase composition on the separation of aromatic compounds was investigated. The results indicated that poly (trimethylol propane triacrylate-co-N-isopropylacrylamide-co-ethylenedimethacrylate) (TMPTA-co-NIPAAm-co-EDMA) monolithic column not only had high porosity and strong rigidity, but also was a promising tool for analyzing small molecule compounds with a short analysis time by controlling the column temperature.

1. Introduction

Monolithic columns were first introduced in 1990s as a new analysis technology applied for the fast separation of biomolecules [1]. During recent years, such polymer separation media have overcome some of the limitations and gradually broaden the range of applications, including solid-phase extraction [2], sample preconcentration [3] and separation analysis [4,5]. Based on the nature of the matrix, monolithic columns are divided into three groups: organic polymer-based, silica-based and organic-silica hybrid monolithic columns. Organic polymer-based monolithic columns have attracted more and more attention recently [6,7] due to their excellent biocompatibility and good stability of pH changes.

Poly (N-isopropylacrylamide) (PNIPAAm) is one of the widely used temperature-sensitive polymers and its aqueous solution can occur phase mutate at nearly 32 °C (LCST) [8–10]. Seino's group [11] prepared poly (N-isopropylacrylamide) (PNIPAAm) grafted silica bead using hyperbranched polysiloxysilane (HBPS) as polymer brush and successfully separated hydrophilic and hydrophobic steroids by the HPLC system. In Dai's study [12], poly (N-isopropylacrylamide) and poly ([2-(methacryloyloxy)ethyl] trimetylammonium chloride) were synthesized and grafted to amino modified silica gel. They achieved baseline separation for lactic acid and creatine phosphate disodium salt. In our laboratory, a temperature-responsive poly (N-isopropylacrylamide-co-N,N′-methylenebisacrylamide) [poly (NIPAAm-co-BIS)] monolith was prepared to separate three aromatic ketones and six steroids with much higher column efficiency [13,14]. All the research mentioned above showed that the retention time of compounds increased with the increase of the temperature. But in Duan's [15] and Zhang's [16] studies, the opposite results were obtained. A view most likely to be accepted so far is that a certain proportion of hydrophobic and hydrophilic groups existed in the molecule, which can interact with water in the intramolecular or intermolecular [17]. Based on Duan's consideration, the length of the polymeric chain and the composition of mobile phase influenced the reversible phase transition at LCST. So the temperature-sensitive mechanism is in the continuously improve.

The trimethylol propane triacrylate (TMPTA) is a multifunctional monomer that can be formed a dense network structure after polymerization reaction [18–20] because of its three vinyl bonds at the end. In this study, a novel poly (TMPTA-co-NIPAAm-co-EDMA) macroporous monolithic column was prepared via in situ free-radical polymerization using TMPTA and NIPAAm as monomers,

* Corresponding authors. Tel.: +86 312 5971107; fax: +86 312 5971107.
E-mail address: lhy1610@126.com (H. Liu).

0021-9673/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2013.11.029

EDMA as crosslinking agent. The influence factors on the preparation and the effect of external temperature on the retention behavior of some small molecules were investigated.

2. Experimental

2.1. Materials

Trimethylol propane triacrylate (TMPTA) was supplied by Tianjin Tianjiao Chemical Co., Ltd. (Tianjin, China). N-isopropylacrylamide (NIPAAm) was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). 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 analysis compounds were provided by the National Institute for the Control of Pharmaceutical and Biological 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. A column heating control system (Waters, America) was used to investigate the influence of temperature on the analysis. 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 micrographs (SEM) of the monolithic columns were carried out on a Hitachi S-4300 SEM instrument (Hitachi High Technologies, Tokyo, Japan).

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

Preparation of the poly (TMPTA-co-NIPAAm-co-EDMA) was carried out by the following method: 0.2 mL TMPTA, 0.1 g NIPAAm, and 0.3 mL EDMA were dissolved in the mixture of 0.5 g PEG and 1.4 mL methanol. Then 0.005 g AIBN was added after the solution was shook for 2 min. The mixture was sonicated for 30 min and bubbled with nitrogen for another 5 min to reinforce dissolve and remove gases. Finally, the reaction solution was poured into a 50 mm × 4.6 mm i.d. stainless steel column that was sealed at the bottom. After being sealed at the top, the stainless steel column was heated up to 70 °C in a water bath for 24 h. After that, the seals were put out and provided with its 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.

2.4. Characterization method

After being rinsing with methanol until a stable baseline was observed, the monolith was pushed out from stainless steel column and then was put in a plate for drying 48 h. Cut a piece of monoliths to grind into powder for performing Fourier transform infrared spectroscopy (FT-IR), which was aimed to confirm the chemical group of the monolith. Cut another piece of monoliths sputtered with gold for carrying out scanning electron microscopy (SEM) to observe the morphology of the monolithic materials.

2.5. Preparation of solutions

All the solutions, including benzene, diphenylamine, biphenyl and phenanthrene were dissolved in the methanol (0.1 mg/mL), which were stored at 4 °C before use.

2.6. HPLC conditions

The HPLC system equipped with a quaternary pump, an autosampler with variable injection capacity from 0.1 to 100 μL and a UV detector. The mobile phase was the mixture of water and methanol, and the UV wavelength was set at 254 nm. The sample injection volume 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 [21–23]. 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^2 is the inner radius of the column. 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 [24].

The retention factor (k) of each aromatic compound on poly (TMPTA-co-NIPAAm-co-EDMA) monoliths at different mobile phase was determined by the equation, $k = (t_R - t_0)/t_0$, where k, t_R,

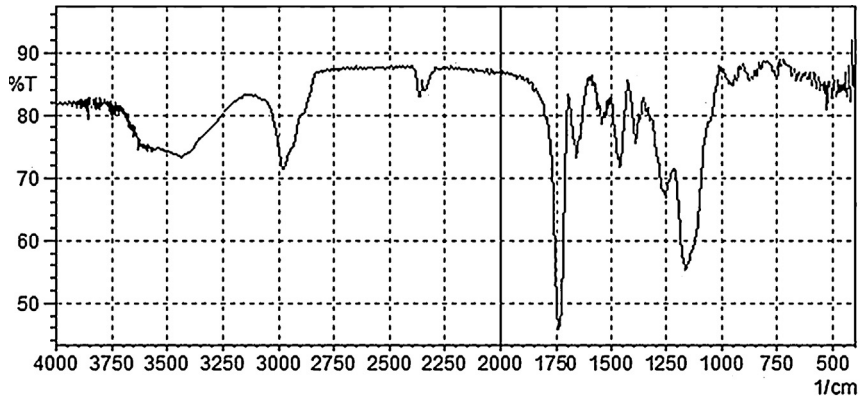

Fig. 1. The FT-IR spectrum of the poly (TMPTA-co-NIPAAm-co-EDMA) monolithic column.