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Characteristics and recognition mechanism of monolithic poly(methacrylic acid-ethylene glycol dimethacrylate) column

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Abstract–Porous polymer monolithic columns have been prepared by the direct free radical copolymerization of methacrylic acid and ethylene glycol dimethacrylate within the confines of a chromatographic column in the presence of toluene-dodecanol as a porogenic solvent. Recognition mechanism and effects of the chromatographic condition such as mobile phase composition, flow rate and temperature on the retention and separation were discussed. The results showed hydrogen-bonding interaction and hydrophobic interaction play an important role in retention and separation in this kind of monolithic column. Compared with traditional particle columns, the monolithic columns showed attractive significant interest because of their ease of preparation, high separation efficiency, low backpressure and fast analysis.

Key words: Monolithic Column, Chromatographic Characteristic, Recognition Mechanism, Theophylline, Caffeine

INTRODUCTION

Monolithic chromatography media represents a novel generation of stationary phases introduced in the last few years providing a chromatography matrix with enhanced mass transfer and hydrodynamic properties [Minakuchi et al., 1996; Tanaka et al., 2001; Nakanishi, 1997]. These kinds of columns have one common characteristic: they are made of one single piece of an adsorbent material (silica or polymer) that fills the entire length of the column [Svec et al., 2000; Motokawa et al., 2002]. Large through-pores are present in this type of stationary phase; it enables mobile phases to flow through the adsorbent with low flow resistance at high flow rates [Jin et al., 2003; Allen and El Rassi, 2004; Chankvetadze et al., 2003]. Because of their ease of preparation, high reproducibility, versatile surface chemistry and rapid mass transport, monolithic stationary phases have become a rapidly expanding field in chromatographic stationary phase preparation in recent years and some have even been commercialized [Kimura et al., 2004; Tanaka et al., 2004; Lubda and Lindner, 2004; Ikegami and Tanaka, 2004]. Some chromatography researchers regard them as fourth-generation chromatography adsorbents [Liu et al., 2005].

Presently, there are two main types of monolithic columns, silica columns [Ishizuka et al., 2002] and porous organic columns [Svec, 2004]. Monolithic columns were introduced first for organic polymers in the late 1980s and early 1990s. Although the column efficiency provided by a polymer monolithic column is generally lower than that by silica monolithic column, the polymeric monolithic column exhibits more potential advantages and a more promising future compared to its silica-based counterparts. This is due to the simpler preparation process, higher efficiency, easier pore size control, and more facile adaptability to adjust column selectivity. Organic polymer monolithic materials could be easily *in situ* synthesized by thermal or irradiation initiating the polymerization, and the pore properties and the surface area of the material could be freely controlled by the composition of the porogenic solvent, crosslinkers and polymerized condition. Poly(methacrylic acid-ethylene glycol dimethacrylate) monolithic material is a widely used monolithic material with methacrylic acid as the acidic monomer and EDMA as the bifunctional crosslinker. This kind of polymer organic material has been used widely in biomedical fields in respect of its biocompatible character [Fan et al., 2004]. Moreover, this kind of polymer monolithic column could be used readily after in situ polymerization. However, there is still a distinct lack of systematic investigation of fabrication and recognition mechanism of monolithic column. In addition, new methods of monolithic columns need to be developed for various different materials due to their special structure.

In this work, we prepared porous organic polymer monolithic columns using methacrylic acid as the acidic monomer and ethylene glycol dimethacrylate as the crosslinker by an in-situ free-radical polymerization "molding" process. The chromatographic characterizations and recognition mechanism of the monolithic columns were tested by a homologous series of xanthine derivatives, theophylline and caffeine. Compared with conventional particle columns, these kinds of monolithic columns have exhibited good stability, ease of regeneration and high-efficiency separation ability.

EXPERIMENTAL

1. Materials

Caffeine and theophylline were obtained from Sigma (ST Louis, MO, USA). The structures of these molecules are shown in Fig. 1. Methacrylic acid (MAA) was purchased from Kanto Chemical Co., Inc. (Japan). Ethylene glycol dimethacrylate (EDMA) was obtained from Tokyo Kasei Kogyo Co., LTD (Tokyo, Japan). α, α'-Azobis (isobutyronitrile) (AIBN) was the product of Junsei Chemical Co.,

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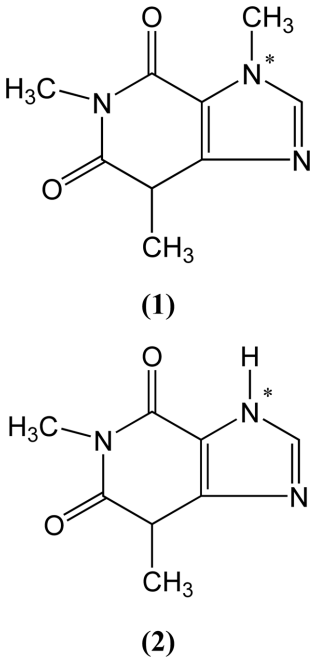

Fig. 1. Molecular structure of caffeine (1) and theophylline (2).

Ltd. (Japan). Toluene was purchased from Orienal Chemical Industries (Japan). Dodecyl alcohol, acetonitrile, chloroform and methanol are all of HPLC grade and from Duksan Pure Chemical Co., LTD (Ansan, Korea). Acetic acid (analytical grade) was purchased from Oriental Chemical Industries (Incheon, Korea). Double distilled water was filtered with 0.45 μm filter membrane before use.

2. Preparation of Monolithic Column

The monolithic columns were prepared by a direct in situ polymerization within stainless steel of a 150 mm×3.9 mm I.D. chromatographic column (Fig. 2). A mixture solution composed of 0.426 ml monomer, 0.940 ml cross-linker (EDMA) and 0.023 g free-radical initiator (AIBN) was dissolved in the porogenic solvents (2.90 ml toluene and 1.00 ml dodecanol). The mixture solution was put into supersonic for 5 min, sparged with helium for 5 min to remove oxygen. The stainless-steel tube sealed at the bottom was filled with the mixture solution and then sealed at the top. The polymerization was performed in a water bath with the temperature maintained at 50 °C for 12 h. After the polymerization, the seals were removed; the column was connected to HPLC pump and washed with tetrahydrofuran and methanol/acetic acid (80 : 20%v/v), respectively, to remove the porogenic solvents and other soluble compounds present in the polymer monolith after the polymerization was completed.

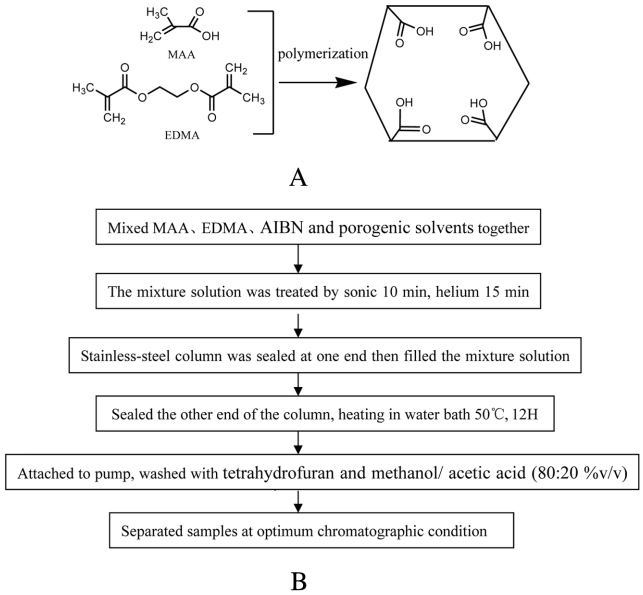

Fig. 2. Schematic illustration of polymerization processes.

3. HPLC Analysis

Separation characteristics of the monolithic column were analyzed by a liquid chromatography system containing Waters 600s Multisolvent Delivery System and a Waters 616 pump (Waters, Milford, MA, USA), Waters 2487 Dual Absorbance UV detector (Waters, Milford, MA, USA) and Rheodyne injection valve (20 ml sample loop). The Millennium 32 software (Waters, Milford, MA, USA) was used as data acquisition system. LiChrospher 100 RP-18 (12 μm) was purchased from Merck (Germany). Acetonitrile was used as mobile phase, UV wavelength at 270 nm.

The separation factor (α) was determined by the following equation:

$$\alpha = k_2/k_1 \quad (1)$$

Where k_2 is the retention factor of the theophylline and k_1 is the retention factor of the caffeine. The retention factor was determined by

$$k = (t_M - t_0)/t_0 \quad (2)$$

where t_M is the retention time of the solute and t_0 is void time of the column. All the procedures were carried out at room temperature.

4. Characterization of Monolithic Stationary Phases

After the chromatographic experiments were completed, the column was washed with methanol/acetic acid (4 : 1 v/v) for 30 min. The bottom column fitting was removed and the monolith inside the column was pushed out of the tube by using the pressure of the methanol mobile phase at a flow-rate of 4 ml/min. The cylindrical monolith was dried under 40 °C for 12 h and cut into pieces with a razor blade. The pore properties and microscopic analysis of the monolith was carried out with an S-4200 Scanning Electron Microscopy (Hitachi, Japan) at 3.0 kV.

RESULTS AND DISCUSSION

1. Comparisons with Traditional Particle Columns

Monolithic columns are free from a densely packed bed of particles. They can be prepared by polymerization of monomers in a column. The process seems to have greater flexibility than packing a column with particles in that a wide range of monomers can be used to prepare a monolith with integrated structures, which can have much higher external porosity than densely packed particles. The main difference of monolithic columns with conventional particle columns is that the monolithic columns have a unique porous structure (Fig. 3). Conventional particle-based supports consist of few micrometer-sized porous particles. Because the pores within the

particles are close to each other, the liquid inside them is stagnant. Therefore, the molecules to be separated are transported to the active sites inside the close pores and back to the mobile phase mainly by diffusion. Since diffusion itself is a rather slow process, especially in the case of large molecules with a low mobility, it enables the mobile phases to flow through the adsorbent with low flow resistance at high flow rates. Monoliths, on the other hand, consist of a single piece of porous material that fills the entire length of the column. The mesopores located on the column skeleton are highly interconnected, forming a network of channels and providing the large surface area needed to achieve sufficient capacity. Meanwhile, the large through-pores are present in this type of stationary phase. It could reduce flow resistance, allowing the use of high flow rates at considerably reduced backpressure. Since the flow of the liquid within the channels is driven by the pressure difference, the molecules to be separated are transported to the active sites located on the surface of the channels by convection, so the monolithic columns can be used under high flow rates to achieve sufficient resolving power. From Fig. 4 we can see theophylline and caffeine could not be separated on conventional Lichrospher 100 RP-18 particle column; however, good separation can be obtained on the monolithic column. Compared with the conventional Lichrospher 100 RP-18 particle column, low backpressures were obtained on the polymer monolithic column at different flow rates. When flow rate was 2.0 mL/min, the column pressure was observed only 8.36 MPa.

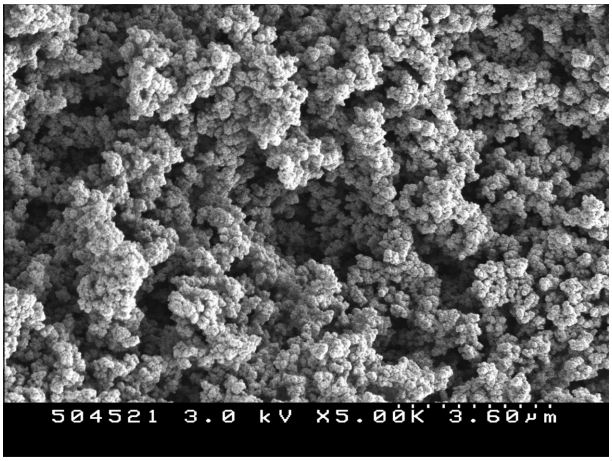

Fig. 3. SEM of the monolithic column.

2. Recognition Mechanism and Effect of the Mobile Phase Composition

The effect of composition of the mobile phase on the separation was investigated by using methanol, water and acetonitrile as mobile phases. The results showed that the caffeine and theophylline cannot be separated by using methanol or water as a mobile phase. The best separation was obtained by using acetonitrile as a mobile phase. The effects of polar additives in the mobile phase were also evaluated with the mixture of acetonitrile-acetic acid as a mobile phase. The results are shown in Figs. 5-6. They show that the increase of the solvent polarity in the mobile phase leads to the decrease of the retention factors of caffeine and theophylline. When only acetonitrile was used as a mobile phase, the best retention factor was attained. These results can be explained by the presence of hydrogen-bonding and hydrophobic interaction between the stationary phase and the sample. In our experiment, methacrylic acid was used as a monomer. Its carboxyl group is the most common hydrogen-bonding and acidic functional group [Wang et al., 2004]. Its hydrophobic polymer bone structure and the acidic pendant groups would appear as an ideal sorbent for basic analytes, which include most of the illicit

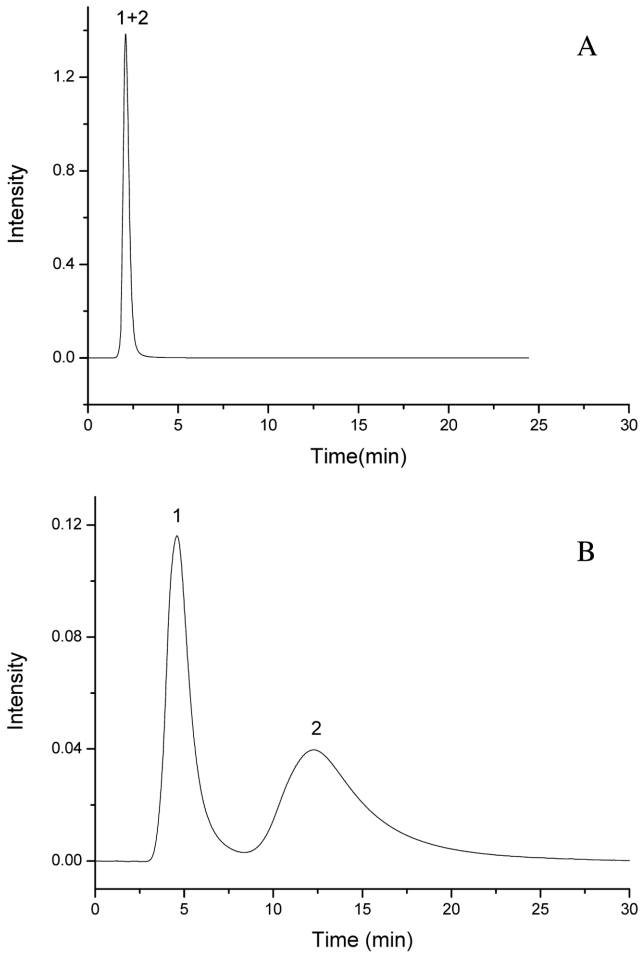

Fig. 4. Chromatograms of caffeine and theophylline on different columns.

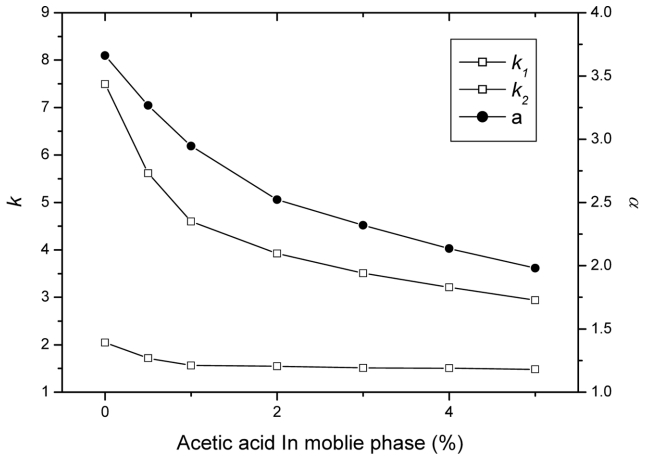

Fig. 5. Effect of mobile phase composition on retention factor and separation factor.

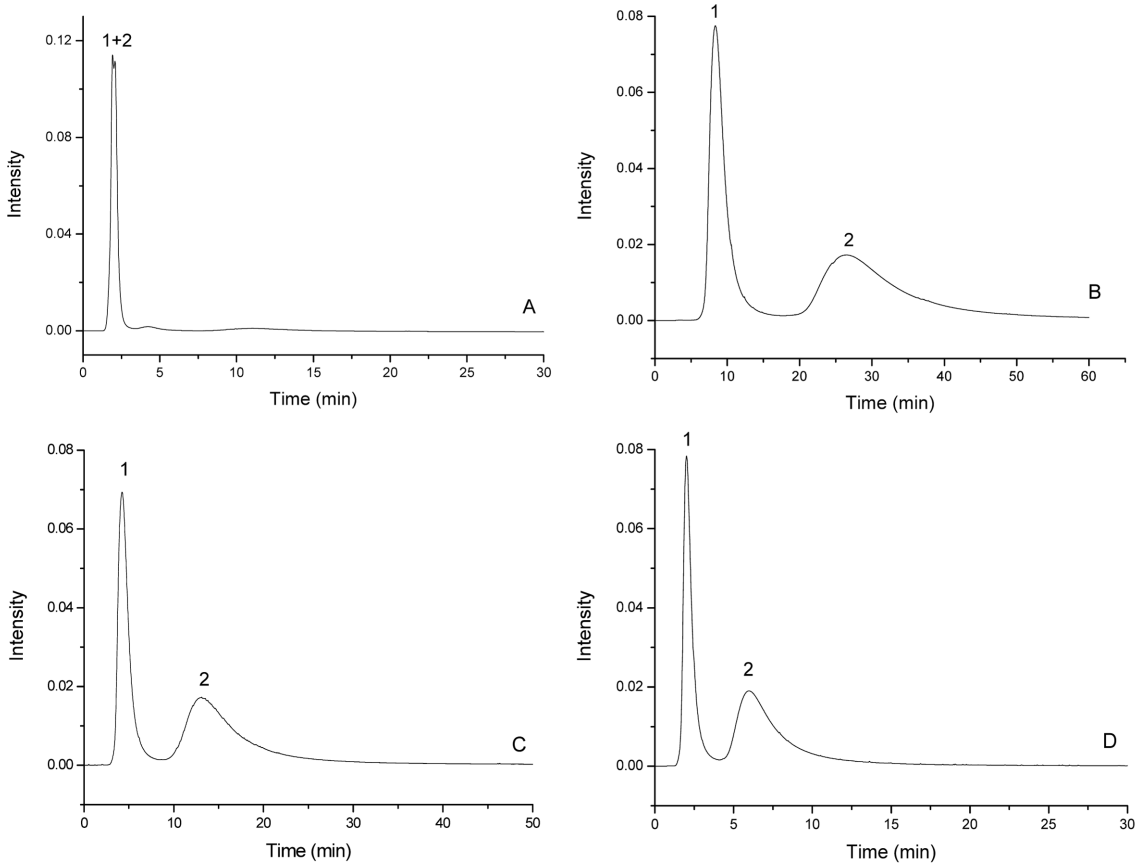

Fig. 6. Chromatograms of caffeine and theophylline at different separation conditions.

drugs and incitants. The basicity of theophylline is stronger (pKb 8.8) than caffeine (pKb 10.4). Because of the weak basicity of caffeine, it can form a weak complex only with MAA than theophylline through hydrogen bond. Therefore, caffeine has a smaller retention time than theophylline on these kinds of monolithic columns.

From the molecular structures of theophylline and caffeine we could see that the only differences between the two templates lie in N*. For caffeine molecules, the hydrogen on N* was placed by methyl, whereas active hydrogen still existed on N* in theophylline. So the amino group in theophylline could form hydrogen bond by the active hydrogen on N* with the carboxylic acid in MAA. And the near oxygen and nitrogen also could form a hydrogen bond with the carboxyl in monomer. Caffeine, on the other hand, since no amino group exists in its molecular structure and the amide group could only form very weak hydrogen bond with the monomer, it is hard to effectively produce molecular recognition. Moreover, since the volume of methyl group is much larger than that of hydrogen, it could block the formation of a hydrogen bond due to a steric hindrance. So theophylline showed a long retention time and a peak tailing on monolithic column. Because the polar additives can cause interference in hydrogen-bonding interactions between the carboxylic acid of MAA in monolithic columns and the functional group of the analytes, so the retention factors of caffeine and theophylline decreased with the increasing of the polarity solvent in the mobile phase. In strong polar solutions such as methanol and water, H^+ destroys the hydrogen-bonding and hydrophobic interaction interactions, so caffeine and theophylline cannot be separated. The hydrogen-bonding and hydrophobic interactions decreased with the increasing of polarity modification and retention factors decreased implying that the hydrogen-bonding interaction and hydrophobic interaction play an important role in the retention and separation.

3. Effect of the Flow Rate on the Separation

Monolithic columns have been studied as materials, having advantages inherent to their network-type one-piece structures. Throughpores provide flow paths through along the column, and the size and density of the macropore network causes the monolithic columns to have a high external porosity, consequently, a large permeability and a low column hydraulic resistance. The second network of mesopores is responsible for the large specific surface area of the monolith, hence for the retention volumes observed for most analytes. For these reasons, monolithic columns are efficient at high flow-rates and can also be used in long connected series, allowing the achievement of very high efficiencies. In this work, the flow rate of the mobile phase was investigated in the range of 0.3-3.0 mL/min. The results are shown in Fig. 7. Although the migration times of the caffeine and theophylline decreased with the increasing of the flow rate, just a slight decrease of the separation efficiency was found when increasing the flow rate. The results show that the dependency of separation efficiency on flow rate is extremely small; therefore, the separation efficiency can be maintained at significantly increased flow rates. This is a typical characteristic of monolithic columns.

4. Effect of Temperature on the Separation

The effects of different temperatures changing from 20 °C to 60 °C

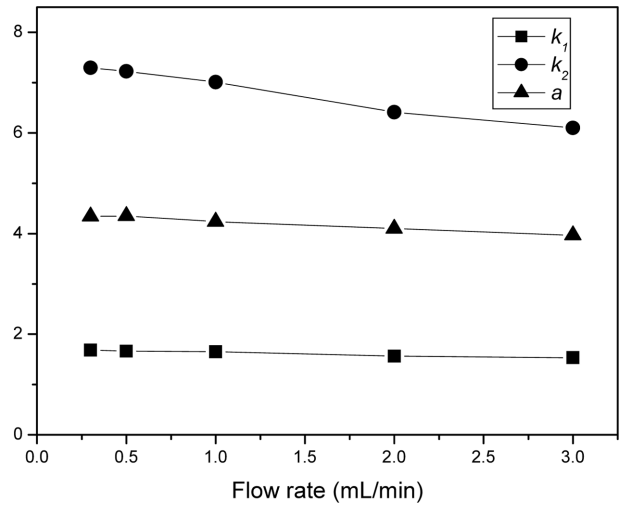

Fig. 7. Effect of flow rate on retention factor and separation factor.

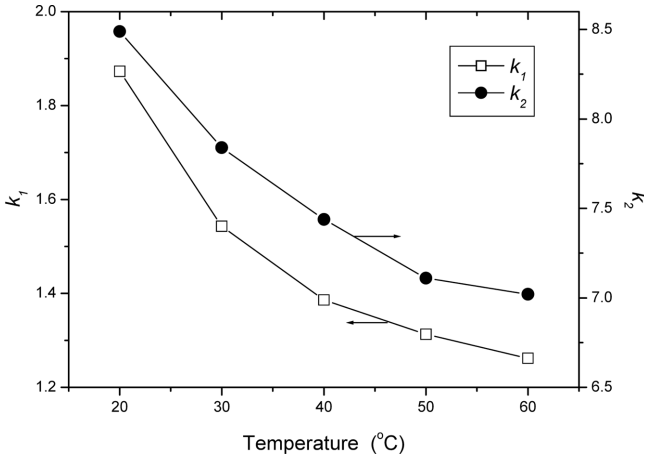

Fig. 8. Effect of different temperatures on the retention factor.

on the separation were also investigated in this paper (Fig. 8). The results show that under higher temperature, both theophylline and caffeine migrate fast, and theophylline changes faster than the caffeine. Both k_1 and k_2 decreased with increasing temperature. It is because analytes have weaker adsorption to the substrate as temperature increases and therefore migrate faster through the monolithic column. Furthermore, the separation factors decreased with increasing elution temperature, due to higher temperature decreasing the interaction between the theophylline and the polymers more than the interaction between caffeine molecule and the polymers. Therefore, a lower temperature will lead to a higher separation.

CONCLUSION

In this work, monolithic columns were prepared by *in situ* therm-initiated polymerization without further processing. The results showed that the dependency of separation efficiency on flow rate is small and hydrogen-bonding and hydrophobic interactions play an important role in the retention and separation. This kind of monolithic column attracts significant interest compared with conventional particle columns because of its ease of preparation, high separation efficiency, and rapid mass transport.

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