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p-tert-Butylcalix[8]arene-bonded silica monoliths for liquid chromatography

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Abstract

Monolithic silica columns have inspired considerable research interests in the separation science because of their unique properties in permeability, mass transfer, efficiency and throughput. In this paper, a chemically *p-tert*-butylcalix[8]arene-bonded silica monolith was prepared as the promising candidate for versatile LC separations. Micrometer-sized macropores and nanometer-sized mesopores in this derivatized silica monolith reduce the diffusion path length and provide both low backpressure and high column efficiencies, leading to high-speed and high-throughput separations. Since *p-tert*-butylcalix[8]arene possesses a π-donors cavity composed of benzene rings while polycyclic aromatic hydrocarbons, anthraquinones, phenol regio isomers and fullerenes are π-systems with appreciable electron affinity, they may have a chance to get involved in forming host–guest inclusion complexes through non-covalent interactions, e.g. hydrophobic and π–π interactions. Compared with RP-18e, the prepared calixarene-bonded monolith exhibited better selectivity to molecules which contains more π-electrons and more condensed cyclic moieties. The column efficiency was about 22 000 plates/m. The calixarene-bonded monolith also showed its good performances in separation of fullerenes and dihydropyridines, indicating a promising approach for purification of fullerenes with high purity from the carbon soot.

Keywords: *p-tert*-Butylcalix[8]arene; Monolithic column; Fullerene; Silica monolith; Calixarene

1. Introduction

Since ordered mesoporous silicates (M41S) were first discovered in the early 1990s, the related materials have sparked a wide spectrum of interests in catalysis, optics, drugs delivery, chemical and biological separations, due to their fascinating structures [1–3]. Among those materials, ordered mesoporous silica may be the most appreciated one that has showed its fantastic properties such as easy of functionality, high surface areas and narrow pore size distributions and attracted much attention especially in separation sciences. Ordered mesoporous silica materials are now available in the forms of control-size uniformed particles, fine powders, and thin films and even in monolith [4–7].

A novel sol–gel process to fabricate well-defined macroporous silica monolith with highly ordered mesopores has been reported by Nakanishi and Tanaka et al. [8–10]. Monolithic silica materials in the shape of cylinders, discs, stacked layers, sponges and chunks for separation sciences have been regarded as promising candidates for versatile applications, due to their unique properties of good permeability, high efficiency, high-speed and high-throughput. The most successful applications of these silica monoliths were stationary phases in high-performance liquid chromatography (HPLC) and capillary electrochromatography (CEC) [11–17]. Monolithic columns are now available commercially and have been used extensively for various chromatographic applications. Surprisingly, most of the related literatures employed either silica-based (normal phase) or C18-bonded silica monolith (reversed-phase). Only a few functionalized silica monoliths for special applications were reported up to the present. To make the monolithic columns versatile, currently, researchers have begun to focus on the functional modification of silica monoliths for fast separations. For this purpose, L-phenylalaninamide-modified and Penicillin G acylase-based silica monoliths were prepared for chiral HPLC

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separations [18,19]. More recently, a molecularly imprinted polymer (MIP) derivatized monolithic silica column was also prepared for enantioseparation applications, exhibiting better efficiency and stability than an organic MIP monolith [20].

Calixarenes and fullerenes are the ideal materials of nanodevices due to their novel electronic and chemical properties [21]. Calixarenes, so-called the third-generation hosts following cyclodextrins and crown ethers, are cavity-shaped cyclic phenol molecules capable of forming host–guest complex with a variety of organic and inorganic guests [22,23]. It has been expected that *p-tert*-butylcalix[8]arene, which has a cavity of depth 3 Å and diameter 11.7 Å, probably exhibited specific properties to form inclusion complexes with π-system-containing molecules such as fullerenes. Now, this expectation has become actual by an accidental finding by Atwood and Shinkai that calix[8]arenes selectively included C60 in carbon soot and formed precipitates with 1:1 stoichiometry [24–26]. Moreover, the ordered nanoarrays of calixarenes and C60/calix[8]arene complex were constructed and characterized on gold surface using electrochemical surface assembling and electrochemical scanning tunneling microscopy (ECSTM) [27].

Recently, some researchers found that calixarene-bonded materials could be used as separation media for polycyclic aromatic hydrocarbons (PAHs) and gestagenes [28–31]. Among the substrates of these calixarene-bonded materials which were either irregular or uniformed polymeric and inorganic particles, the porous spherical silica was the most favorite candidate because of its high surface areas and versatile modification strategies. However, when these beads were packed into columns for liquid chromatographic preparation or analysis of the target compounds, some inherent limitations such as the slow diffusional mass transfer, large void volume and high backpressure always led to low efficiency and time-consuming separations [31,32].

Monolithic stationary phases were expected to improve the separation and enable direct analysis with high-speed and high performance. Here, we report a new type of *p-tert*-butylcalix[8]arene-bonded monolithic silica column with macro- and mesopores. This calixarene-bonded monolith was characterized, exhibited its good properties and versatile applications in LC separation.

2. Experimental

2.1. Materials and chemicals

Tetramethoxysilane (TMOS) and poly(ethylene glycol) (PEG, MW = 10 000) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Ethyl bromoacetate, 3-aminopropyltriethoxysilane (APTES) and *p-tert*-butylcalix[8]arene were purchased from TCI (Tokyo, Japan). Aloe emodin, emodin, chrysophanol and physcin were purchased from Sigma (St. Louis, MO, USA). Amilodipine, nifedipine, nitrendipine and nimodipine were kindly donated by North China Pharmaceutical Co. (Shijiazhuang, China). HPLC-grade methanol, acetonitrile and hexane were purchased from Fisher (Fair Lawn, NJ, USA). DI water (resistivity 18.2 MΩ) was prepared from a Millipore system (Milford, MA, USA). Other reagents were obtained from various commercial sources and were analytical grade unless otherwise specified.

2.2. Preparation of monolithic silica column

The silica monolith for liquid chromatography was prepared using the well-known sol–gel reaction [8–10]. Briefly, a mixture of TMOS (14 mL), PEG 10 000 (2.0 g) and urea (2.4 g) in 0.01 M acetic acid (30 mL) was stirred at 0 °C for 30 min to form a homogenous solution. The resultant solution was poured into a PTFE tube (100 mm × 5 mm I.D.) and to gel at 40 °C for 18 h. The silica monolith was obtained and then aged at 40 °C for 8 h. The wet gel was transferred to an autoclave and immersed into appropriate amount of external solution (0.1M aqueous ammonium hydroxide). The autoclave was heated up to 120 °C for 12 h to form mesopores in the silica gel. After that, the silica rod was washed with *N*,*N*-dimethylformamide and then dried at 60–100 °C for 12 h. Subsequently, the monolithic silica gel was heated at 700 °C for 2 h to decompose organic materials and stabilize the hydrophilic surface. The obtained silica monolith was refluxed in 6 M HCl aqueous solution for 24 h and finally dried at 110 °C for 4 h.

2.3. Preparation of *p-tert-butylcalix[8]arene-bonded silica monolith*

Preparation of *p-tert*-butylcalix[8]arene-bonded monolith is illustrated in Fig. 1. Octa(chloroformylmethoxy)calix[8]arene (**4**) was derived from *p-tert*-butylcalix[8]arene (**1**) as described elsewhere [32]. Firstly, *p-tert*-butylcalix[8]arene octaester (**2**) was prepared by refluxing *p-tert*-butylcalix[8]arene (1.3 g, 1 mmol) with excessive ethyl bromoacetate (3.6 mL, 30 mmol) and NaH (1.1 g, 30 mmol) in 50 mL tetrahydrofuran for 12 h in the presence of nitrogen gas (the yield was 72.4% for this step). The product was then hydrolyzed by refluxing with a mixture of 10 mL NaOH (1 M) and 40 mL ethanol for 12 h. The yield of *p-tert*-butylcalix[8]arene carboxylic acid (**3**) was about 92.6%. Amount of 0.85 g (0.5 mmol) of **3** was dissolved in 10 mL of dried toluene, refluxed with 10 mL of thionyl chloride for 2 h in the presence of nitrogen gas. The excessive thionyl chloride was then removed in vacuum and the product **4** was used in subsequent synthesis without further purification.

The activated monolithic silica rod was fitted into a stainless steel column (100 mm × 4.6 mm I.D.) with inert material such as epoxy and polytetrafluoroethylene. The column was put into a 90 °C thermostat then connected to a LC pump. A solution of 10% 3-aminopropyltriethoxysilane in anhydrous toluene (50 mL) was pumped through the column at a flow rate of 0.04 mL/min for 18 h to obtain the 3-aminopropyltriethoxysilylated silica monolith (**5**). To this column, a solution of **4** in anhydrous toluene (5 mM, 50 mL) was then pumped through under the same conditions. Finally, this calixarene-bonded silica monolith was sequently washed with toluene and methanol until no unreacted reagents were detected in the eluent.