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Ethane-bridged hybrid monoliths with well-defined mesoporosity and great stability for high-performance peptide separation

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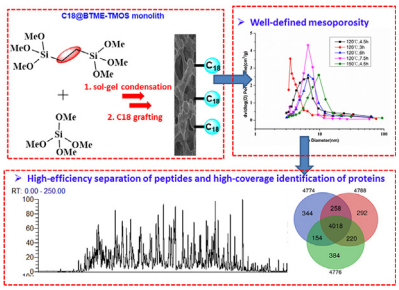
HIGHLIGHTS

- A hybrid monolith was prepared by bridged organosilane.
- The monolith has well-defined mesopore structure and good stability.
- Peptides were separated with high performance and good reproducibility.
- Complex samples were separated and identified by MS successfully.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel kind of hybrid monolith with well-defined mesopore structure was prepared based on sol-gel condensation of 1, 2-bis(trimethoxysilyl)ethane (BTME) and tetramethoxysilane. Compared with terminal organosiloxanes used for preparation of conventional hybrid monoliths, BTME, as an ethane-bridged alkoxysilane precursor, could not only maintain the uniformity of pore size distribution, but also improve the chemical stability of the monolith via Si-C bonds in the framework. Owing to the controllable mesoporous structure and good stability, the monolithic column was used for the separation of peptides with half peak width less than 6 s and the run-to-run and column-to-column relative standard deviations (RSD) for the retention time of five standard peptides less than 2.5%, showing narrow peak width and good reproducibility. Moreover, the separation performance could be well maintained even after washed by the mobile phase with pH 11.0 at 50 °C. Furthermore, 100 cm-length monolithic column was prepared and successfully used for nanoRPLC-ESI-MS/MS analysis of HeLa cell lysate digests, and 5670 proteins corresponding to 37574 peptides were identified from 750 ng of the sample, showing great promising of "single-shot" large-scale in-depth proteomic research.

1. Introduction

Monolithic materials have been widely used as the stationary phases for chromatographic separation [1–4], especially for peptide separation in the field of proteomics because they can be prepared with meter-scale length but low backpressure to further

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improve separation performance followed by mass spectrometry identification [5]. Generally, based on the nature of the matrix, monoliths can be classified into three categories: organic polymer-based [6–8], silica-based [9–11] and organic-silica hybrid monolith [12–14]. Organic polymer-based monoliths own the advantages of ease of preparation and surface chemistry modification, and high chemical stability over a wide pH range. However, the lack of mesopores leads to low surface area which is unfavorable to improve sample loading capacity and the inhomogeneity of pores distribution has a bad influence on separation performance. In comparison, silica monoliths, prepared by hydrolytic polycondensation of tetraalkoxysilanes, have high efficiency in separation of both small and large molecules, owing to their well-defined mesopore size distribution [15], but their ease of hydrolysis of Si-O-Si linkage in acid mobile phase or dissolution in alkaline mobile phase has negative effects on the stability and reproducibility of separation. In recent few years, organic-silica hybrid monolith has been developed to combine the merits of both organic polymer-based and silica-based monoliths, such as ease of preparation, good chemical and mechanical stability.

Hybrid monoliths are mainly synthetized by acid/base-catalyzed sol-gel co-condensation of terminal organofunctional silanes with tetraalkoxysilanes [16–20]. The introduction of terminal organosilanes facilates surface functionalization and facilitates the chemical stability via the additional Si-C bond. However, it has disrupting effect on the degree of mesoscopic order [21,22]. Another method is the “one-pot” approach developed by Zou et al. [23,24], via free radical copolymerization of vinyl-organic monomers with hydrolyzed tetramethoxysilane (TMOS) and vinyltrimethoxysilane (VTMS). Although various kinds of functionalized hybrid monoliths [25–27] could be designed by changing available vinyl-organic monomers, the free radical copolymerization faces the challenge of uncontrolled pore structure [28]. Furthermore, Zou et al. [29–31] have developed another strategy to synthesize hybrid monolith by copolymerization of organic monomers with polyhedral oligomeric silsesquioxane (POSS). Due to the incorporation of POSS, the monoliths show good mechanical and pH stability. The homogeneous macropores structure can be obtained when using “ring-opening” [29] or “click chemistry” [30,32] polymerization, but absence of mesopores across the monolith might hinder its further application in proteomics, especially in separation of peptides. It is well known that mesopore size and distribution play important roles in loading capacity, separation efficiency. Nevertheless, the hybrid monoliths prepared by these three methods are lack of uniform mesopore structure. Therefore, to further improve the separation performance, the mesopore size distribution should be well-controlled for the hybrid monolithic materials.

The main aim of this work is to develop a new kind of hybrid monolith with well-defined mesopore structure and excellent stability for liquid chromatography. Recently, organic bridged-bonded alkoxysilane precursors ($(R'O)_3Si-R-Si(OR')_3$) with self-oriented organic functional groups (R) have been developed to synthesize hybrid silica materials [33–35]. In this work, the new monolith has been prepared based on the sol-gel condensation of 1,2-bis(trimethoxysilyl)ethane (BTME) and tetramethoxysilane, followed by pore-expansion, calcination and C18-derivatization. The introduction of ethane-bridged silica precursor BTME could not only maintain the order of pore structures, but also improve the chemical stability of the monolithic materials via Si-C bonds in the framework. The obtained hybrid monolith with tailorable mesopore size and good stability was successfully used for chromatographic separation of peptides with high efficiency and good reproducibility. Finally, the monolith was further successfully applied for proteomic analysis of complex sample extracted from *HeLa* cell lysates by nanoRPLC-ESI-MS/MS.

2. Experimental section

2.1. Materials

1,2-Bis(trimethoxysilyl)ethane (BTME, >97.0%) was purchased from TCI (Shanghai, China), Tetramethoxysilane (TMOS, 98%) was purchased from J&K Scientific Ltd. (Beijing, China). Poly(ethylene glycol) (Mr 10^4, PEG), trichloro(octadecyl)silane (≥90), trifluoroacetic acid (≥99%) formic acid (~98%) and urea (≥99.5%) were all obtained from Sigma-Aldrich (St. Louis, MO). Ammonium formate (≥98%) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Toluene (Kaixin, Tianjin, China) was refluxed (110 °C) over sodium and distilled (136 °C). The five peptides, R-V-Y-H-P-I (883.1Da), R-V-Y-V-H-P-F (917.1Da), A-P-G-D-R-I-Y-V-H-P-F (1271.4Da), D-R-V-Y-V-H-P-F-H-L (1282.5Da), and D-R-V-Y-I-H-P-F-H-L (1296.5Da) were all purchased from ChinaPeptides Co., Ltd. (Shanghai, China). Methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Deionized water was used in the experiments (Millipore, Milford, MA). Fused-silica capillaries (75 μm i.d. × 375 μm o.d.) were purchased from.

2.2. Apparatus

Scanning electron micrographic (SEM) images were obtained using a JSM-7800F field-emission scanning electron microscope (JEOL, Tokyo, Japan). For the nitrogen adsorption-desorption measurements (QuadraSorb SI4, U.S.A.), the materials were pretreated at 150 °C for 12 h under vacuum. Chromatographic measurements were performed on a micropump LC system (Michrom Bioresources, CA, US) with a UV detector K-2501 (Knauer, Berlin, Germany). The flow rate of 100–500 nL/min was adjusted by the splitting capillary. All chromatographic data were collected and evaluated using HW-2000 software (Beijing Huayanglimin Scientific Instrument Ltd., Beijing, China). The retention factor (k) was defined as $(t_r-t_0)/t_0$, where t_r and t_0 represent the retention times of the peptides and unretained compound, respectively.

2.3. Synthesis of ethane-bridged hybrid monolith

Firstly, the capillary was pretreated to expose the maximum number of silanol groups at the inner surface of the silica capillary. It was flushed with 1 M NaOH for 6 h, washed by water for 30 min, rinsed with 1 M HCl for 3 h, flushed with water and methanol successively, and then dried at 120 °C under nitrogen atmosphere overnight. The ethane-bridged hybrid silica monolithic column (BTME-TMOS monolith) was prepared by sol-gel condensation of 1,2-Bis(trimethoxysilyl)ethane (BTME) and tetramethoxysilane (TMOS). Briefly, 100 μL BTME and 400 μL TMOS were added into 0.01 M acetic acid (2.0 mL) with 0.17 g PEG and 0.16 g urea and stirred at 0 °C for 40 min to form homogeneous solution. Then the solution was induced into the pretreated capillary with a syringe. With both ends sealed by rubber, the capillary was heated at 40 °C for 24 h. Then the obtained hybrid silica monolithic capillary column was treated at 120–150 °C for 4.5–7.5 h with ammonia generated by the hydrolysis of urea to complete the formation of mesopores, followed by washed with water and methanol to remove the porogens and unreacted reagents. After drying slowly at 50 °C, the calcination of the monolith was carried out at 200 °C for 25 h under air.

For monolithic material modifications, 10% (v/v) trichloro(octadecyl)silane (dissolved in anhydrous toluene) was pumped through the BTME-TMOS monolith at room temperature for 24 h via nitrogen pressure. Finally, the monolith was washed with toluene and methanol successively.

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