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Preparation of open tubular capillary columns by *in situ* ring-opening polymerization and their applications in cLC-MS/MS analysis of tryptic digest

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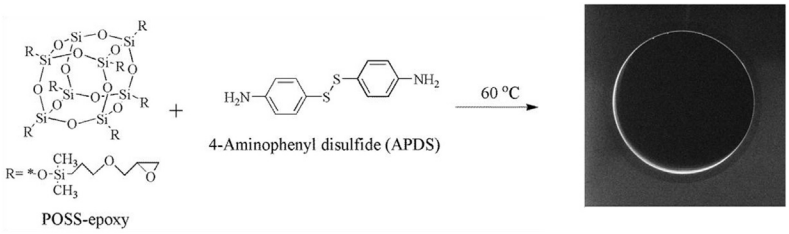
HIGHLIGHTS

- It was the first time to prepare OT column by ring-opening polymerization.
- The ratio of ethanol/H_2O at 13/1 (v/v) was used in the synthesis of the OT phases.
- The OT column was successfully applied in cLC-MS/MS analysis of tryptic digest.

GRAPHICAL ABSTRACT



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ABSTRACT

An open tubular (OT) column (25 μm i.d.) was prepared by *in situ* ring-opening polymerization of octaglycidyldimethylsilyl polyhedral oligomeric silsesquioxanes (POSS-epoxy) with 4-aminophenyl disulfide (APDS) in a binary porogenic system of ethanol/H_2O. It was found that porogenic composition played an important role in the formation of OT stationary phases. The ratio of ethanol/H_2O at 6/1 (v/v) would lead to the fabrication of hybrid monoliths, while the ratio of ethanol/H_2O at 13/1 (v/v) would result in the synthesis of OT phases. In addition, the effects of precursor content and reaction duration on the thickness of OT stationary phases were investigated. Either lower precursor content or shorter reaction duration would produce thinner layer of OT column. The repeatability of OT columns was evaluated through relative standard deviation (RSD%) with benzene as the analyte. The run-to-run, column-to-column and batch-to-batch repeatabilities were 1.7%, 4.8% and 5.6%, respectively, exhibiting satisfactory repeatability of the OT column. Then tryptic digest of mouse liver proteins was used to evaluate the performance of the resulting OT columns (25 μm i.d. × 2.5 m in length) by cLC-MS/MS analysis, demonstrating their potential in proteome analysis.

1. Introduction

Over the past two decades, mass spectrometry has become one of the most important analytical techniques [1–4]. Due to high resolving capacity, high sensitivity, low sample and mobile phase

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consumption, capillary liquid chromatography-tandem mass spectrometry (cLC-MS/MS) has been widely employed in the analysis of complicated biological samples, particularly in the proteomics research [5,6]. As the "heart" of chromatographic technique, chromatographic columns play vitally important roles in highly efficient separation of complex samples. To date, three kinds of capillary columns including packed column [7–9], monolithic columns [8,10–13] and open-tubular (OT) columns [14–19] are the most common tools for separation of complicated biosamples by cLC-MS/MS analysis.

OT columns are open tubes with the stationary phase immobilized as a coating onto the inner wall of the capillary [20]. They have attracted great attention in the separation of large biomolecules on account of the low column backpressure [21,22]. Several preparation techniques [23–27] such as dynamic coating, static coating and *in situ* polymerization were developed in the manufacture of OT columns, which exhibited different advantages and disadvantages. For instance, although OT phases could be easily prepared with dynamic or static coating, they were apt to 'bleed' from the stationary phase, plugging columns or even damaging detectors. By comparison, the OT phases fabricated via *in situ* polymerization exhibited excellent stability as the layer was chemically bonded onto the capillary wall [24,28,29]. Free radical polymerization [30–34] and sol-gel chemistry [26,35] were two common strategies for the fabrication of bonded OT columns. For example, poly(styrene-divinylbenzene) (PS-DVB) OT column could be directly prepared by thermally initiated free radical polymerization with ethanol as the sole porogenic solvent. Karger's group [31] successfully applied the PS-DVB OT column in proteomics by cLC-MS/MS analysis, and 3046 unique peptides covering 566 distinct *Methanosarcina acetivorans* proteins were identified from 50 ng in-gel tryptic digest sample. Nesterenko and coworkers [32] developed an automated "in-capillary" UV-initiated free radical polymerization technique for the fabrication of OT columns with precisely controlled layer thickness and length. In the work, they investigated the relationship between exposure times, intensity, multiple exposures and layer thickness. The resulting OT columns showed excellent longitudinal homogeneity. Additionally, Desmet et al. [36] synthesized mesoporous silica layer with a thickness up to 550 nm in 5 μm i.d. capillaries by sol–gel chemistry. The 2.5 m-long OT columns exhibited excellent column efficiencies of 600,000 and 1,000,000 plates for retained and unretained compounds, respectively. These results could trigger interests in development of novel fabrication approaches for OT columns in cLC.

Ring-opening polymerization has been adopted in the fabrication of hybrid monoliths [37]. However, to the best of our knowledge, there were not any reports on OT columns prepared by ring-opening polymerization. Herein, an OT column was fabricated by utilizing *in situ* ring-opening polymerization of octaglycidyldimethylsilyl polyhedral oligomeric silsesquioxanes (POSS-epoxy) with 4-aminophenyl disulfide (APDS) (Scheme 1). The morphology of the resulting poly(POSS-APDS) layers was investigated by scanning electron microscopy (SEM), and an OT column (25 μm i.d. × 2.5 m in length) was estimated by cLC–MS/MS analysis of mouse liver tryptic digest.

2. Experimental section

2.1. Materialsh

Polyimide coated fused-silica capillaries (25, 50 and 200 μm i.d.) were provided by Yongnian Optical Fiber Factory (Hebei, China). (3-Aminopropyl) trimethoxysilane (APTMS), POSS-epoxy, APDS, trypsin and formic acid were purchased from Sigma (St Louis, MO, USA). Dithiothreitol (DTT) and iodoacetamide (IAA) were obtained from Sino-American Biotechnology Corporation (Beijing, China). Acetonitrile (ACN, HPLC grade) was bought from Merck (Darmstadt, Germany). Doubly deionized water was prepared with a Milli-Q system (Milli-pore, MA, USA). Methanol, ethanol, 1-propanol, 2-propanol, 1,4-butanediol, PEG400, PEG10000, sodium hydroxide (NaOH) and hydrochloric acid (HCl) were the products of Tianjin Kermel Chemical Plant (Tianjin, China).

2.2. Preparation of poly(POSS-APDS) OT columns

Prior to preparation of OT columns, the inner wall of fused-silica capillary was pretreated with APTMS for anchoring the OT layer. Briefly, the capillary was rinsed using 0.1 mol L^{-1} NaOH, H_2O, 0.1 mol L^{-1} HCl, H_2O and methanol for 2 h, successively. Then the capillary was filled with APTMS solution in methanol (50%, v/v), sealed with rubbers at both ends and submerged in water bath at 50 °C for 12 h. Finally, the capillary was washed with methanol and blown with nitrogen stream at 2 MPa for 6 h.

The synthesis of poly(POSS-APDS) OT columns was summarized in Scheme 1. Briefly, POSS-epoxy and APDS (proportions in accordance with Table 1) was dissolved in a mixture of ethanol and H_2O, and then sonicated for 15 min. The resulting mixture was introduced into the pretreated capillary under nitrogen pressure of 3 MPa and heated at 60 °C for 12 (columns III-V), 9.5 (column VI), 9 (column VII) or 6 h (column VIII). The obtained columns were then flushed with methanol to remove the solvents, unreacted monomers as well as the oligomers.

Additionally, multistep polymerization was explored to fabricate the OT columns. In brief, the column VI was blown with nitrogen stream at 2 MPa for 6 h. The mixture of POSS-epoxy and APDS with the same proportion as column VI (Table 1) was introduced into the column VI and heated at 60 °C for 9.5 h. The resulting column was assigned as column IX. Similarly, column X was fabricated by repeating the above procedures for three times.

The remaining mixtures in the vials were also heated with the columns to fabricate the bulk material. The material was washed with ethanol to remove the residuals and dried under vacuum at 80 °C for 24 h. The resulting material was further used for physical characterization.

2.3. Physical characterization

The SEM images were obtained from Orion NanoFab helium ion microscope (HIM, Germany). Energy-dispersive X-ray spectrum (EDX) was recorded using the secondary electron imaging mode on a JEOL JSM-5600 scanning electron microscope (JEOL, Tokyo, Japan). Fourier-transformed infrared spectroscopy (FT-IR) was performed on a Thermo Nicolet 380 spectrometer (Nicolet, Wisconsin, USA). KBr pellets, comprised of 1 mg dry material and 100 mg KBr, were obtained by powder compressing machine. The thermogravimetric analysis (TGA) experiment was carried out on a Setsys 16/18 (Setaram, Caluire, France) from 40 to 700 °C at a ramp rate of 10 °C min^{-1}. Water contact angle was measured by a JC2000C 147 machine (Powereach, Shanghai, China). Nitrogen adsorption/desorption measurements were performed at 77 K on a Quadrasorb SI surface area analyzer (Quantachrome, Boynton Beach, USA). The total surface area of the bulk stationary phase was calculated using the Brunauer-Emmett-Teller (BET) model.

2.4. Tryptic digest of proteins

The sample was prepared according to the procedures published previously [38]. One milligram of mouse liver protein was dissolved in 1 mL denaturing buffer, which contained 8 mol L^{-1} urea and 0.1 mol L^{-1} ammonium bicarbonate. After the addition of 20 μL DTT