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Short communication

Bridged polysilsesquioxane-based wide-bore monolithic capillary columns for hydrophilic interaction chromatography

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ABSTRACT

The synthesis and characterization of large-bore silica-based monolithic capillary columns (0.32 mm × 150 mm) are presented. Columns were prepared by acidic hydrolysis of a mixture containing tetramethoxysilane (TMOS) and 1,2-bis(trimethoxysilyl)ethane (BTME) in different molar ratios in the presence of polyethylene glycol and urea. The monoliths were modified by zwitterionic monomer [2-(methacryloyloxy)ethyl]-dimethyl-(3-sulfopropyl)-ammonium hydroxide via 3-(trimethoxysilyl)propyl methacrylate. Prepared stationary phases were evaluated by scanning electron microscopy and chromatographic separation of nucleobases and their derivatives in the HILIC mode. The best chromatographic results were obtained with the column prepared from the reaction mixture containing BTME and TMOS in a 1:4 molar ratio. The permeability of such column reached 1.68×10^{-14} m^2 and the efficiency, expressed as a height equivalent of the theoretical plate, did not exceed 10.5 μm for the tested compounds. The columns were successfully applied to HILIC separation of native and labeled oligosaccharides and glycans released from bovine ribonuclease B and human immunoglobulin G.

1. Introduction

Since the group of Nakanishi and Tanaka published their concept of preparation of silica-based monolithic columns [1], many other researchers have presented works dealing with synthesis of silica-based monoliths, especially in a capillary format. Detailed information on preparation and development of the silica-based monolithic columns can be found in several comprehensive reviews published during past decades [2–4]. It should be noted that a vast majority of laboratory-synthesized capillary columns was characterized under CEC conditions and only a few preparation protocols allowed synthesis of stable and efficient silica-based monolithic columns suitable for LC.

Generally, alkoxysilane precursors prevail over other compounds used. Probably the best known silica-based monolithic columns were prepared by acidic hydrolysis of tetramethoxysilane (TMOS) in the presence of polyethylene glycol 10,000 followed by post-modification reaction to obtain desired stationary phases, e.g., C18 [5]. In order to simplify the column preparation, post-modification reaction was replaced by incorporation of functional groups directly into the monolithic backbone. This can be achieved using desirable alkyltrialkoxysilane itself or in a mixture with alkoxysilanes subjected to acidic or basic hydrolysis. These columns are called hybrid organic-inorganic monolithic materials, which can be prepared via several approaches such as ring-opening polymerization [6–9], radical polymerization [10–12] or thiol-ene reaction [13]. A few years ago, so called “one-pot” process was introduced where alkyltrialkoxysilanes containing multiple/double bonds are used together with organic monomers as precursors. In this case, sol-gel reaction proceeds in a first step followed by radical polymerization.

Protocols for synthesis of silica-based monolithic capillaries have been gradually improved over the time in order to obtain highly efficient columns for LC. Columns prepared from pure TMOS or mixture of TMOS and methyltrimethoxysilane (MTMS) allowed efficient LC separation of small molecules in RP as well as HILIC separation modes. For example, minimum achieved plate height (H) reached a value of less than 6 μm under RP [14] as well as HILIC separation conditions [15]. Similar separation efficiency was also achieved for columns prepared by thiol-ene reaction [13], “one-pot” preparation procedure [16], and ring-opening polymerization reactions [17,18] where precursors based on polyhedral oligomeric silsesquioxanes were employed.

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The silica-based monolithic columns are usually prepared in a capillary with i.d. of up to 0.1 mm. However, the fabrication of silica-based monolithic materials in a larger diameter capillary is still challenging due to the shrinkage of the monolith resulting in cracks of the monolithic scaffold and detachment of the monolithic rod from the capillary wall. This is particularly true for monoliths prepared by acidic hydrolysis of TMOS.

The synthesis of silica-based monolithic columns in wider capillaries can be beneficial for chromatographic separation, e.g., to lower the effects of extra-column band broadening on the column efficiency, to minimize extra-column effects or to enable the injection of larger sample volumes in a stronger solvent. Incorporation of the organic part into the monolithic backbone can also improve the mechanical stability of silica-based monolithic capillary columns as confirmed by Motokawa et al. [19]. An addition of alkyltrialkoxysilane (MTMS) to the reaction mixture was presented as an effective approach to prepare monolithic capillary columns with i.d. of 0.2 mm [19] and more even up to 0.53 mm [20]. On the other hand, the monoliths prepared from pure MTMS in the 0.53-mm i.d. capillaries showed lower separation efficiency [21,22] and the performance of these columns was not comparable to columns prepared in a narrower fused silica capillary.

Another precursor suitable for synthesis of silica-based monolithic materials is 1,2-bis(trimethoxysilyl)ethane (BTME). BTME belongs to the group of bridged polysilsesquioxanes with organic moieties built into a siloxane matrix by hydrolytically stable covalent carbon-silicon bonds. Therefore, BTME reacts in the same way as TMOS by "sol-gel" reaction and the synthesized materials have similar hydrophilicity as TMOS-based monoliths. Furthermore, sufficient concentration of silanols for functionalization is available on the monolithic surface. On the contrary, monoliths synthesized only from MTMS, where organic moiety is present as the chain-end group, showed a hydrophobicity comparable to the commercial C8 columns [21,22]. Although monolithic materials based on BTME were synthesized and characterized more than 10 years ago [23–26], their utilization in chromatographic separation has not been fully explored.

Presented work covers synthesis of wide-bore silica-based monolithic columns in a 0.32–0.53 mm i.d. capillary from a reaction mixture containing BTME and TMOS in various molar ratios. The columns were modified to the zwitterionic stationary phase via the two-step approach published earlier [15] and characterized by scanning electron microscopy (SEM). Chromatographic performance of synthesized columns was evaluated in the HILIC separation mode.

2. Experimental

All chemicals and standards of the highest purity were purchased from Sigma–Aldrich (Prague, Czech Republic). Fused silica capillaries (0.32, 0.45, and 0.53 mm i.d.) were purchased from Agilent Technologies (Waldbronn, Germany).

2.1. Preparation of monolithic capillary columns

Fused silica capillaries were treated by 1 M NaOH and 1 M HCl for 1 h at 40 °C. Then, the capillaries were flushed by distilled water, ethanol, and dried at 100 °C under the stream of nitrogen, cut to 250 mm long pieces and filled by a mixture containing alkoxysilane precursors. Both ends of each column were sealed by a methane-oxygen micro flame burner and the capillaries were placed into the GC oven for 16 h at 45 °C. Finally, the temperature was raised with gradient of 0.5 °C/min up to 120 °C and kept constant for 2.5 h. After cooling, the column ends were cut off and the prepared columns were rinsed by distilled water until neutral reaction of the eluent. The water was then replaced by absolute ethanol (30 column volumes) and the columns were allowed to dry at room temperature for 24 h. Calcination of silica monoliths was performed by heating from 35 °C up to 120 °C (a gradient of 0.5 °C/min), then raised up to 320 °C (a gradient of 1.0 °C/min), and followed by a final isothermal step at 320 °C for 8 h. After cooling to ambient temperature, the pure silica monolithic column was cut down and modified to the zwitterionic stationary phase using a [2-(methacryloyloxy)ethyl]-dimethyl-(3-sulfopropyl)-ammonium hydroxide monomer according to the previously published protocol [15].

Reaction mixtures used for preparation of silica monolithic columns contained pure BTME and mixtures of BTME and TMOS in a molar ratio of 1:1, 1:2 or 1:4, where a total amount of alkoxysilica precursors in the reaction mixture was 3.36 mmol. For example, mixture used for preparation of 1:4 BTME:TMOS monolith consisted of 0.11 g PEG and 0.225 g urea in 2.5 ml of 0.01 M acetic acid, 169 μl of BTME and 400 μl of TMOS. The mixture was stirred for 15 min at room temperature and degassed before filling the capillary.

2.2. Instrumentation

Chromatographic separations were performed using a Dionex UltiMate 3000 Nano LC System where UV detection was employed for isocratic separations of nucleic acid bases, nucleosides, and 2-deoxynucleosides. An ESI-TOF-MS (Bruker maXis impact) were employed for detection of native and labeled glycans separated in a gradient mode. Separation conditions are shown under individual figures.

Standard oligosaccharides and glycans released from bovine ribonuclease B and human immunoglobulin G were labeled by reductive amination using (2-aminoethyl)-trimethylammonium chloride (AETMA) according to the previously published protocol [27].

3. Results and discussion

The silica-based monolithic capillary columns have been conventionally synthesized by sol-gel polymerization of tetraalkoxysilanes namely TMOS and its mixtures with MTMS. However, a number of different synthesis approaches employing, e.g., hybrid organic-inorganic precursors has been developed in the last years [4]. In this paper, we present successful synthesis of silica-based monolithic columns in the capillary with the i.d. of 0.32–0.53 mm using a reaction mixture consisting of bridged silsesquioxane, BTME, and TMOS as precursors, and their application for chromatographic separation in the HILIC mode.

3.1. Characterization of monolithic columns by SEM

In order to prepare a mechanically stable silica-based monolithic column in a 0.32-mm i.d. capillary the composition of the reaction mixture was optimized, especially the molar ratio of precursors, BTME and TMOS. The total amount of alkoxysilica precursors in the reaction mixture was estimated as 3.36 mmol in 2.5 ml of 0.01 M acetic acid, while the amounts of other compounds in the mixture were kept constant, for details see *Experimental section*. The morphology of synthesized silica-based monoliths was investigated by scanning electron microscopy (SEM) and the micrographs of the capillary cross-sections and details of the monolithic matrix are shown in Fig. 1. The SEM micrographs clearly show the effect of the composition of the reaction mixture on the stability and structure of the synthesized materials. Monolith prepared only from BTME showed highest density and shrinkage. It occupied only around 50% of the capillary space after thermal treatment