



Tuning preparation conditions towards optimized separation performance of thermally polymerized organo-silica monolithic columns in capillary liquid chromatography



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ABSTRACT

Tuning of preparation conditions, such as variations in the amount of a porogen, concentration of an aqueous acid catalyst, and adjustment in polymerization temperature and time, towards optimized chromatographic performance of thermally polymerized monolithic capillaries prepared from 3-(methacryloyloxy)propyltrimethoxysilane has been carried out. Performance of capillary columns in reversed-phase liquid chromatography was assessed utilizing various sets of solutes. Results describing hydrophobicity, steric selectivity, and extent of hydrogen bonding enabled comparison of performance of hybrid monolithic columns prepared under thermal (TSG) and photopolymerized (PSG) conditions. Reduced amounts of porogen in the polymerization mixture, and prolonged reaction times were necessary for the preparation of monolithic columns with enhanced retention and column efficiency that reached to 111,000 plates/m for alkylbenzenes with shorter alkyl chains. Both increased concentration of catalyst and higher temperature resulted in faster polymerization but inevitably in insufficient time for pore formation. Thermally polymerized monoliths produced surfaces, which were slightly more hydrophobic (a methylene selectivity of 1.28 ± 0.002 TSG vs 1.20 ± 0.002 PSG), with reduced number of residual silanols (a caffeine/phenol selectivity of 0.13 ± 0.001 TSG vs 0.17 ± 0.003 PSG). However, steric selectivity of 1.70 ± 0.01 was the same for both types of columns. The batch-to-batch repeatability was better using thermal initiation compared to monolithic columns prepared under photopolymerized conditions. RSD for retention factor of benzene was 3.7% for TSG capillaries ($n = 42$) vs. 6.6% for PSG capillaries ($n = 18$). A similar trend was observed for columns prepared within the same batch.

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1. Introduction

High performance liquid chromatography (HPLC) is the most widely utilized analytical separation technique due to its universal applicability to quantitative and qualitative analysis of a wide range of compounds. This technique can be used for separation of analytes varying greatly in polarity and charge. In contrast to gas chromatography, limited thermal stability and volatility of analytes is of less concern in HPLC. Large numbers of columns, commercial and custom-made, are available enabling these separations, even though combination of columns with diverse chemistry might be required for complex mixtures.

In recent years the quest for acceleration of analyses, while preventing the loss in column efficiency, led to development of highly permeable monolithic stationary phases. This concept was first envisioned by Mould and Syngé in 1952 [1]. As with traditional particle-packed columns, developing formulas for preparation of monolithic columns based on organic polymers [2–5] and silica [6–10] attracted the most attention. Silica-based monolithic columns are prepared using sol-gel technology whereas free radical polymerization of suitable organic monomer is most often used for formation of organic polymer monoliths. Structure porosity and surface chemistry have been shown to control chromatographic performance of both types of monolithic columns. The size of through-pores and silica skeletons is controlled in silica-based monoliths independently by controlling composition of reaction mixture, and the concentration of silane and polyethylene glycol. Furthermore, thermal post-gelation treatment in basic conditions

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leads to formation of mesopores within the silica skeletons [11,12]. In the case of polymer monoliths, porosity is optimized by adjusting the percentage of the porogen and cross-linking monomer in the polymerization mixture, as well as temperature and duration of the polymerization reaction [13,14].

A fair number of manuscripts were devoted to the preparation of hybrid organo-silica monoliths [15–20]. These monoliths have also been subject of our attention, specifically those prepared using 3-(methacryloyloxy)propyltrimethoxysilane. In our early work inspired by research of Dulay et al. [15–17], we have optimized conditions for the preparation of photopolymerized sol-gel (PSG) monoliths, and evaluated their chromatographic properties utilizing capillary liquid chromatography [18]. We have reported that these stationary phases have free silanols as well as organic moieties available on the pore surface, and therefore could be further modified with various organic functionalities via silanization or graft polymerization. In addition, these materials exhibited an interesting potential for the separation of large molecules such as proteins. Hence, these separation media were promising in proteomics applications.

In this manuscript we extended our early findings mainly by switching from photoinitiation to thermally initiated polymerization. We assumed that temperature will simultaneously increase the rate of hydrolysis and polycondensation of methoxysilanes as well as the rate of initiation of free radical polymerization of methacrylate groups of the starting monomer. This should ultimately result into formation of monoliths with different porosity and surface chemistry compared to photopolymerized monoliths. Further, we have evaluated the effect of variations in the amount of the porogen, the concentration of the aqueous acid catalyst, polymerization temperature, and time on the column permeability, retention, and efficiency. Subsequently, several sets of solutes were separated to assess chromatographic performance of thermally (TSG) and photopolymerized (PSG) sol-gel monoliths.

2. Material and methods

2.1. Chemicals and materials

3-(Methacryloyloxy)propyltrimethoxysilane (MPTMS, 98%), 2,2'-azobisisobutyronitrile (AIBN, 98%), anhydrous toluene, together with high purity HPLC solvents, methanol, ethanol, tetrahydrofuran, acetonitrile, and probe compounds of at least 98.5% purity were purchased from Sigma Aldrich (St. Louis, MO).

2.2. Preparation of sol-gel monoliths

Monolithic capillaries were prepared by mixing 575 μ L of 3-(methacryloyloxy) propyltrimethoxysilane and 100 μ L of 0.15 mol/L HCl for 30 min. Simultaneously, in a separate dark brown vial, 30 mg AIBN (5% w/v) was stirred with 420 μ L toluene (70% v/v). Next, 180 μ L MPTMS/HCl solution was added to the solution of initiator in toluene. The solution was vortexed for 3 min, and purged with nitrogen for 30 s. UV-transparent fused silica capillaries (25 cm) were filled with the final mixture using compressed nitrogen, were plugged with a rubber septa and were either heated (TSG, 80 °C) or irradiated (PSG, 365 nm, 900 mJ/cm²). The temperature in the instrument used for irradiation was not controlled but did not exceed 29 °C, i.e. a temperature that does not start thermally initiated polymerization. After the polymerization was completed, the capillaries were rinsed with toluene for removal of unpolymerized components, and their length reduced to 18 cm.

2.3. Instrumentation

Thermal polymerizations were carried out in an Isotemp oven (Fisher Scientific, Pittsburgh, PA). Irradiation of the capillaries was performed using the XL-1500A UV crosslinker with six 365 nm tubes (both Spectroline, Westbury, NY). Chromatographic performance was evaluated using a 1200 Series capillary pump (Agilent Technologies, Wilmington, DE), connected to an Agilent 35900E A/D interface, a Rheodyne MXP7980-000 injector (Chrom Tech Inc., Apple Valley, MN) equipped with 8 nL injection loop (injection time 0.1 s), and a Spectra System UV 2000 detector (Thermo Separation Products) equipped with a 50 μ m I.D. UV transparent capillary as the detection cell. Fourier transform infrared spectroscopy (FT-IR) was carried out using a 100 Series FT-IR spectrometer (Perkin Elmer) equipped with attenuated total reflectance (ATR). For this purpose monoliths were prepared in 4 mL vials at 80 °C, washed several times with ethanol to remove unreacted components, and dried in the vacuum oven to eliminate residual solvent and moisture. Liquid samples were analyzed without any processing.

2.4. Chromatographic conditions

For separation of alkylbenzenes under isocratic conditions an aqueous acetonitrile solution ranging from 35 to 60% was employed as a mobile phase. The solution of a mixture of alkylbenzenes consisted of uracil (0.7 mg/mL), benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, pentylbenzene, and hexylbenzene (each 20 μ L/mL in 80% aqueous acetonitrile). Analytes were eluted at a flow rate of 1 μ L/min and were detected at a wavelength of 214 nm. Each injection was repeated 5 times. For evaluation of the presence of free silanols on the pore surface in the monoliths, separation of a mixture of uracil (0.5 mg/mL), caffeine (3.3 mg/mL), phloroglucinol (3.2 mg/mL), resorcinol (2.9 mg/mL), and phenol (3.0 mg/mL) in 20% aqueous acetonitrile was carried out at a flow rate of 1 μ L/min, and detection wavelength of 214 nm. For evaluation of the steric selectivity, a mixture of thiourea (1.2 mg/mL), *o*-terphenyl (3.6 mg/mL), and triphenylene (0.4 mg/mL) in a mobile phase consisting of 70% aqueous acetonitrile was separated at a flow rate of 1.5 μ L/min and detected at a wavelength of 254 nm.

3. Results and discussion

3.1. Acid catalyzed hydrolysis/polycondensation and free radical polymerization of MPTMS

Our initial work aimed at rapid preparation of photopolymerized sol-gel hybrid organo-silica monoliths to obtain separation media for applications in reversed-phase capillary liquid chromatography [18]. This study expands the early effort aiming at tuning preparation conditions towards optimized separation performance of thermally polymerized sol-gel monoliths, and further comparison of chromatographic properties of both types of columns.

Organo-silica hybrid monoliths were prepared using 3-(methacryloyloxy)propyltrimethoxysilane as the single monomer, hydrochloric acid as an aqueous catalyst, toluene as a porogen, and 2,2'-azobisisobutyronitrile as commonly available initiator. The MPTMS monomer used as the starting material contains both inorganic and organic functionality, thus enabling, under specific reaction conditions, preparation of hybrid monoliths containing siloxane and carbon-carbon chains. It is known that in the presence of an aqueous acid the methoxy groups of the methoxysilane (Si-OCH₃) undergo simultaneous hydrolysis and polycondensation

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