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Facile preparation of organic-silica hybrid monolith for capillary hydrophilic liquid chromatography based on “thiol-ene” click chemistry

Ming-Luan Chen[1], Jun Zhang[1], Zheng Zhang, Bi-Feng Yuan, Qiong-Wei Yu, Yu-Qi Feng*

Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), Department of Chemistry, Wuhan University, Wuhan 430072, China

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ABSTRACT

In this work, a one-step approach to facile preparation of organic–inorganic hybrid monoliths was successfully developed. After vinyl-end organic monomers and azobisisobutyronitrile (AIBN) were mixed with hydrolyzed tetramethoxysilane (TMOS) and 3-mercaptopropyltrimethoxysilane (MPTMS), the homogeneous mixture was introduced into a fused-silica capillary for simultaneous polycondensation and “thiol-ene” click reaction to form the organic-silica hybrid monoliths. By employing this strategy, two types of organic-silica hybrid monoliths with positively charged quaternary ammonium and amide groups were prepared, respectively. The functional groups were successfully introduced onto the monoliths during the sol–gel process with “thiol-ene” click reaction, which was demonstrated by ζ-potential assessment, energy dispersive X-ray spectroscopy (EDX), and Fourier transform infrared (FT-IR) spectroscopy. The porous structure of the prepared monolithic columns was examined by scanning electron microscopy (SEM), nitrogen adsorption–desorption measurement, and mercury intrusion porosimetry. These results indicate the prepared organic-silica hybrid monoliths possess homogeneous column bed, large specific surface area, good mechanical stability, and excellent permeability. The prepared monolithic columns were then applied for anion-exchange/hydrophilic interaction liquid chromatography. Different types of analytes, including benzoic acids, inorganic ions, nucleosides, and nucleotides, were well separated with high column efficiency around 80,000–130,000 plates/m. Taken together, we present a facile and universal strategy to prepare organic-silica hybrid monoliths with a variety of organic monomers using one-step approach.

1. Introduction

Since hydrophilic interaction chromatography (HILIC) was proposed and investigated systematically by Alpert in 1990 [1], it has become a complementary alternative to reversed-phase chromatography (RPLC) in the separation of polar compounds [2,3]. Up to date, hydrophilic monolithic columns have been successfully applied in the separation of various types of analytes, such as peptides [4–6], nucleic acid bases, nucleosides, and nucleotides [7–9], oligodeoxynucleotides [10], amino acids [8], sugar derivatives [6,11] and some small polar molecules [12–14]. Because of their fast mass transfer, good permeability and high column efficiency, silica-based monolithic columns have been attracting wide attentions on capillary HILIC (*c*HILIC) [5,15]. Nevertheless, the commercial hydrophilic silane monomers are not available, and the preparation process of silica monolithic columns is typically complicated. Particularly, the tedious post-treatment, including aging, drying and chemical modification, results in week-long preparation time with poor reproducibility, which seriously restricts the applications of silica monoliths in HILIC [15,16].

Sol–gel technique has been applied to prepare silica-based monoliths over the past decades [17–21]. Conventionally, the sol–gel procedure involves two harmonious steps, the hydrolysis of silanes with organic moieties and tetra-alkoxysilanes (either tetramethoxysilane (TMOS) or tetraethoxysilane (TEOS)), and the polycondensation of silanes to form uniform and mechanically stable silica network with expected functional groups on the monolithic surface. The use of a mixture of tetra-alkoxysilanes and silanes with organic moieties instead of tetra-alkoxysilanes alone can minimize the shrinkage of the silica gel network. The mesopores can be formed just by the hydrolysis of urea at 120 °C [22]. Yet, in order to achieve a satisfactory domain size, it needs at least 24 h preparation time with slow condensation rate and precise thermal treatment. The subsequent aging process frequently causes shrinkage of the silica monolithic framework since the aqueous solvent

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* Corresponding author. Tel.: +86 27 68755595; fax: +86 27 68755595.
E-mail address: yqfeng@whu.edu.cn (Y.-Q. Feng).

[1] These authors contributed equally to this work.

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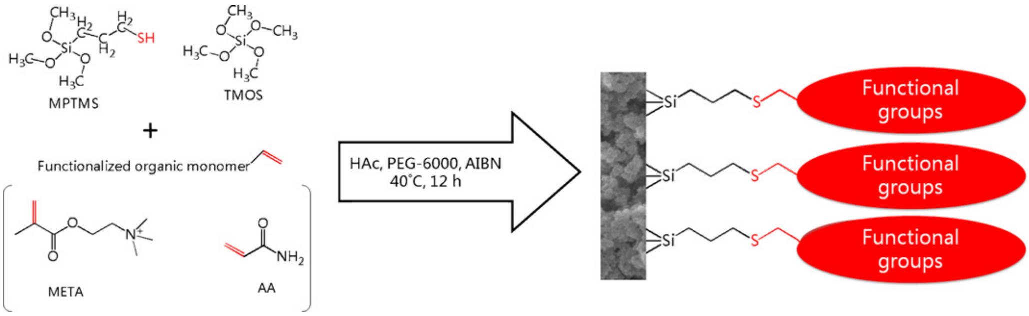

Fig. 1. Scheme of the preparation of organic-silica hybrid monolith.

will be easily evaporated at 120 °C. In addition, because of the lack of commercially available hydrophilic silane monomers, the design and synthesis of silanes with desirable hydrophilic functionalities are indispensable.

In 2001, Dulay and co-workers employed a photopolymerized sol–gel method to prepare a hydrophobic organic-silica hybrid monolithic column [21]. The prepared monolithic column exhibited stable porous structure and good separation resolution towards polycyclic aromatic hydrocarbons. Recently, a “one-pot” method has been emerged as an alternative for the preparation of organic-silica hybrid monoliths, which realizes the functionalization via free radical polymerization of vinyl groups during the formation of silica skeleton [5,23]. Apparently, the utilization of various functional organic monomers can avoid tedious synthesis of functional silanes and the combination of simultaneous polycondensation and free radical polymerization leads to simple preparation process. Nevertheless, the prepared monoliths still need extra 3 h aging in ammonia solution. Besides, the self-polymerization of functional organic monomers frequently affects the domain size, which may cause bad permeability and low column efficiency. Therefore, a facile approach for preparation of the organic-silica hybrid monoliths with appropriate domain size and desirable functional group will highly facilitate the preparation process.

The “thiol-ene” click reaction has many merits, such as simplicity, high efficiency and high conversion under mild conditions [24–26], thus such approach has been utilized in the preparation of both particulate-packed columns and polymer-based monoliths [27–30]. Recently, the “thiol-ene” click reaction was applied in the preparation of hydrophilic silica stationary phase by incorporating zwitterionic cysteine on vinyl-end silica particulates [27]. The resulting stationary phase showed high hydrophilicity. Subsequently, it was employed in the post modification of vinyl-end silica hybrid monoliths [31,32]. Yet, in these reports, a limited range of thiol-ene organic monomers was employed with a traditional two-step thermal-treatment process.

Herein, we report a universal method for the facile preparation of organic-silica hybrid monoliths that overcomes the shortcomings associated with conventional two-step sol–gel synthesis and the free radical polymerization of vinyl groups. When the vinyl-end organic monomers, azobisisobutyronitrile (AIBN), TMOS and 3-mercaptopropyltrimethoxysilane (MPTMS) were mixed together, the homogeneous mixture was introduced into a fused-silica capillary for simultaneous polycondensation and “thiol-ene” click reaction to form organic-silica hybrid monoliths at 40 °C for 12 h (Fig. 1). With the proposed method, two types of organic-silica hybrid monolithic columns were successfully prepared using [2-(methacryloyloxy)ethyl]trimethylammonium (META) and acrylamide (AA), respectively. The characterizations of the prepared monolithic columns show large specific surface area, good mechanical stability, and excellent permeability. With proved anion-exchange/hydrophilic interaction chromatographic retention mechanism, the prepared monolithic columns can well separate different types of analytes, including benzoic acids, inorganic ions, nucleosides, and nucleotides with high column efficiency around 80,000–130,000 plates/m. These results demonstrate that the strategy developed here facilitates the one-step preparation of monoliths carrying various functional groups.

2. Materials and methods

2.1. Chemicals and buffers

META (80 wt% in H_2O, containing 600 ppm monomethyl ether hydroquinone as the inhibitor) and AA were purchased from Acros (New Jersey, USA). To remove inhibitors, META was extracted with 10% aqueous sodium hydroxide and water; after drying over $MgSO_4$, they were filtered and distilled under reduced pressure. AIBN and poly(ethylene glycol) with the molecular weight of 6000 (PEG-6000) were all purchased from Shanghai Chemical Reagent Corporation (Shanghai, China). AIBN was purified by recrystallization from ethanol at 40 °C. TMOS and MPTMS were purchased from Wuhan University Silicone New Material (Wuhan, China). HPLC-grade acetonitrile (ACN) were obtained from TEDIA Company (Ohio, USA). The water used throughout all experiments was purified using a Milli-Q apparatus (Millipore, Bradford, USA). The fused-silica capillaries with 75 μm i.d., 360 μm o.d. were purchased from Yongnian Optic Fiber Plant (Hebei, China).

Adenine (A), uracil (U), cytosine (C), thymine (T), guanine (G), 2′-deoxycytidine (dC), 2′-deoxyguanosine (dG), 2′-deoxyadenosine (dA), and 2′-deoxycytidine (dC) were purchased from Shanghai Kayon Biological Technology (Shanghai, China). Adenosine 5′-monophosphate (AMP), adenosine 5′-diphosphate (ADP), and adenosine 5′-triphosphate (ATP), uridine 5′-triphosphate (UTP), cytidine 5′-triphosphate (CTP), and guanosine 5′-triphosphate (GTP) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Thiourea, benzoic acids, inorganic ions, including bromide (KBr), nitrate ($AlNO_3$), iodide (KIO_3), and other chemicals of analytical grade used in this experiment were purchased from Shanghai Chemical (Shanghai, China). The standard solution of each analyte was prepared at 1.0 mg/mL in H_2O and stored at −4 °C in the dark.

2.2. Preparation of organic-silica hybrid monolithic columns

The fused-silica capillaries were washed with 1 mol/L NaOH (2 h), H_2O (30 min), 1 mol/L HCl (1 h), H_2O (30 min), and methanol (30 min) successively to activate the silanol groups on the wall. Then the capillaries were allowed to dry under nitrogen flow at 160 °C for 5 h.

A polycondensation mixture containing acetic acid (HAc) (0.01 M, 500 mg, 67.5% w/w_{total}), PEG-6000 (25 mg, 3.4% w/w_{total}), TMOS (185 mg, 25.0% w/w_{total}), MPTMS (15 mg, 2.0% w/w_{total}), META (16 mg, 2.2% w/w_{total}) and AIBN (1 mg, 6.7% w/w_{MPTMS}) was