



# One-pot preparation of glutathione–silica hybrid monolith for mixed-mode capillary liquid chromatography based on “thiol-ene” click chemistry



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## ABSTRACT

A novel glutathione (GSH)–silica hybrid monolithic column synthesized via a combination of thiol-ene click reaction and one-pot process was described, where thiol-end GSH organic monomer and 2,2-azobisisobutyronitrile (AIBN) were mixed with hydrolyzed tetramethoxysilane (TMOS) and  $\gamma$ -methacryloxypropyltrimethoxysilane ( $\gamma$ -MAPS) and then introduced into a fused-silica capillary for simultaneous polycondensation and “thiol-ene” click reaction to form the GSH–silica hybrid monolith. The effects of the molar ratio of TMOS/ $\gamma$ -MAPS, the amount of GSH, and the volume of porogen on the morphology, permeability and pore properties of the prepared GSH–silica hybrid monoliths were studied in detail. A uniform monolithic network with high porosity was obtained. A series of test compounds including alkylbenzenes, amides, and anilines were used to evaluate the retention behaviors of the GSH–silica hybrid monolithic column. The results demonstrated that the prepared GSH–silica hybrid monolith exhibited multiple interactions including hydrophobicity, hydrophilicity, as well as cation exchange interaction. The run-to-run, column-to-column and batch-to-batch reproducibilities of the GSH–silica hybrid monolith for phenols' retention were satisfactory with the relative standard deviations (RSDs) less than 1.3% ( $n=5$ ), 2.6% ( $n=3$ ) and 3.2% ( $n=3$ ), respectively, indicating the effectiveness and practicability of the proposed method. In addition, the GSH–silica hybrid monolith was applied to the separation of nucleotides, peptides and protein tryptic digests, respectively. The successful applications suggested the potential of the GSH–silica hybrid monolith in complex sample analysis.

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## 1. Introduction

Monolithic materials as stationary phases have been developed as an alternative to the classic particles packing materials for chromatographic separations in high performance liquid chromatography (HPLC), capillary liquid chromatography (cLC) and capillary electrochromatography (CEC) [1–3], enzyme immobilization [4] and solid-phase microextraction (SPME) [5] in the past two decades. The most interest in monolithic columns is attributed to

their excellent permeability, fast mass transfer kinetics and ease of preparation compared to traditional packed columns [6,7]. Based on the nature of the matrix chemistry, monolithic columns can be mainly classified into two types: the organic polymer-based [8–10] and the inorganic silica-based monolithic columns [11–13]. Generally speaking, the organic monolithic columns can provide good pH stability and great flexibility to tune the chemical properties of monoliths by using a variety of functional monomers and crosslinkers [14]. However, due to its deficiencies of mechanical stability and desirable porous structure, the organic monolith still has limitation in some applications. In contrast, despite the high surface area and high mechanical stability, the surface functionalization of silica-based monolithic columns is labor-intensive and time-consuming. As an alternative, the third type of organic–inorganic hybrid monolithic columns first emerged in 2004 [15] and has

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gained great popularity in recent years since it combines the merits of the organic polymer-based and silica-based monoliths [16–20]. In particular, it is noteworthy that Zou's group [21–23] recently proposed a facile one-pot approach for the synthesis of organic–inorganic hybrid monolithic columns, where the organic functional monomers can be directly incorporated into the inorganic silanes, and then polycondensation and polymerization are carried out in one pot by a stepwise reaction temperature. Apparently, the utilization of various organic monomers in one-pot process can avoid tedious synthesis of functional silanes, opening a new way for obtaining diverse organic–inorganic hybrid monolithic columns with desirable organic functionalities [24–27]. Nevertheless, self-polymerization of functional organic monomers frequently affects the domain size, which may cause bed permeability and low column efficiency. Therefore, a facile approach for preparation of the organic–inorganic hybrid monolithic column with appropriate domain size and desirable functional group will highly facilitate the preparation process.

Recently, an important segment of “click chemistry”, i.e. radical-based thiol-ene reaction, has been attracting great interest since it possesses several advantages such as simplicity, high efficiency, high selectivity and high conversion under mild conditions [28–31]. The thioether linkage formed serves as a strong and stable covalent bond, which is able to withstand harsh conditions. Thiol-ene reaction has been widely employed for the preparation of chromatographic stationary phases including particle-packed columns and polymer-based monoliths [32–36]. Besides, silica-based monolithic column with post-modification of hydrophilic *n*-octadecanethiol via thiol-ene click chemistry has also been reported [37]. In these monoliths, however, a limited range of thiol-ene organic monomers was employed with a traditional two-step process. Until recently, Yao's group [38] and Feng's group [39] successively developed an one-pot approach for the preparation of organic–inorganic hybrid monoliths via thiol-ene click chemistry, in which tetramethoxysilane (TMOS) and 3-mercaptopropyltrimethoxysilane (MPTMS) were adopted as co-precursors and vinyl-containing organic monomers were used as functional moieties. Nevertheless, it is still theoretically unavoidable that the self-polymerization of vinyl-containing organic monomers occurred.

Reduced glutathione (GSH), as a hydrophilic tripeptide composed of glutamine, cysteine, and glycine, possesses a pendant sulfhydryl group and can be reacted with vinyl-containing monomers via “thiol-ene” click chemistry. Furthermore, it contains two free carboxylic acid groups and one amino group, and should exhibit ion-exchange characteristics if immobilized. Lately, a novel type of zwitterionic stationary phase was prepared by covalently bonding GSH on silica gel via click chemistry, which exhibited good hydrophilicity and cation-exchange characteristics [33]. However, to the best of our knowledge, no studies on one-pot process in combination with click chemistry for the preparation of GSH–silica hybrid monolithic column have been reported so far.

Herein, we reported a facile one-pot approach in combination with “thiol-ene” click reaction for the synthesis of the GSH–silica hybrid monolithic columns by using the hydrolyzed TMOS and  $\gamma$ -methacryloxypropyltrimethoxysilane ( $\gamma$ -MAPS) as co-precursors and GSH as functionalized organic monomer, respectively. The synthetic procedure was as simple and efficient as *in situ* polymerization of polymer-based monolith without any special handling. The influences of the ratio of TMOS to  $\gamma$ -MAPS, the amount of GSH, and the content of porogenic solvent on the morphology, permeability and column performances of the hybrid monoliths were investigated in detail. The applications of the newly designed hybrid separation media to separate a series of small molecules and tryptic digestion of protein were also discussed in this work.

## 2. Experimental

### 2.1. Materials

TMOS and  $\gamma$ -MAPS were products of Chemical Factory of Wuhan University (Wuhan, China). Ethylene glycol (EG), reduced GSH and poly (ethylene glycol) (PEG,  $M_n = 10,000$ ) were purchased from Alfa Aesar (Ward Hill, MA, USA). AIBN was obtained from Tianjin Chemistry Reagent Factory (Tianjin, China) and recrystallized with methanol (MeOH) prior to use. Sequencing-grade modified trypsin (TPCK-trypsin) was from Promega (Madison, WI, USA). Bovine serum albumin (BSA) was purchased from Beijing Dingguo Co. Ltd (Beijing, China). Five peptides (Tyr–Gly–Gly (YGG), Cys–Tyr–Ile–Gln–Asn–Cys–Pro–Leu–Gly (CYIQNCPLG), Tyr–Gly–Gly–Phe–Leu (YGGFL), Arg–Ser–Gly–Phe–Tyr (RSGFY), and His–Cys–Lys–Phe–Trp–Trp (HCKFWW)) were purchased from Shanghai Apeptide Co. Ltd (Shanghai, China). Nucleotides including thymidine monophosphate (TMP), uridine monophosphate (UMP), deoxyadenosine monophosphate (dAMP), guanosine monophosphate (GMP) and cytidine monophosphate (CMP) were obtained from Sigma (St. Louis, MO, USA). Alkylbenzenes, thiourea, anilines, phenols and HPLC-grade acetonitrile (ACN) were obtained from Sinopharm Chemical Reagent (Shanghai, China). All other chemicals were of analytical grade or better. Deionized water was prepared with a Milli-Q water purification system (Millipore, Milford, MA). Capillaries with 370  $\mu\text{m}$  o.d.  $\times$  75  $\mu\text{m}$  i.d. were the products of Yongnian Optic Fiber Plant (Hebei, China).

### 2.2. Instruments

All of hybrid monolithic capillaries with a total length of 50 cm (effective length 25 cm) were used unless otherwise stated. All chromatographic experiments were performed on a TriSep-2100 pressurized capillary electrochromatography (pCEC) instrument (this instrument can also be utilized as capillary liquid chromatography system, Unimicro Technologies, Pleasanton, CA, USA) as described previously [17]. A flow rate of 0.05 mL/min was used unless otherwise stated and the UV absorbance was monitored at 214 nm. Samples were injected through an injection valve with an internal 2  $\mu\text{L}$  sample loop. A four-port splitter was set between the injection valve and the monolithic column to split the flow into a desirable and stable flow rate. Since the splitting ratio was set at 400:1, the actual injection volume was about 5 nL. Scanning electron micrographs (SEM) of the hybrid monolithic column was carried out on a XL-30E scanning electron microscope (Philips, The Netherlands). The adsorption–desorption isotherms of liquid nitrogen were measured by using physisorption analyzer (Micromeritics ASAP 2010 porosimeter, USA). Fourier transform infrared (FT-IR) spectra of the monolithic materials were recorded using the AVATAR 360 FT-IR spectrophotometer (Nicolet, Waltham, MA, USA), where 3 mg powder sample was mixed with 100 mg KBr.

### 2.3. Preparation of the GSH–silica hybrid monolithic column

In order to covalently anchor the silica matrix to the capillary wall, the inner surface of the capillary was treated with a vinyl silanizing agent according to the previous procedure [40]. The schematic preparation of the GSH–silica hybrid monolithic column was illustrated in Fig. 1. A prehydrolyzed mixture was prepared by mixing and stirring acetic acid (0.01 M, 5 mL), PEG10 000 (540 mg), TMOS (1.8 mL), and  $\gamma$ -MAPS (0.5 mL) for 1 h at ice bath to form a homogeneous solution. Then, 30 mg of GSH and 1 wt% AIBN (1 mg) dissolved with 80  $\mu\text{L}$  EG were added into 0.5 mL of the resulting hydrolyzed mixture and then sonicated for 20 min. Afterward, the mixture was injected into the pretreated capillary to an appropriate length with a syringe. When both ends of the capillary were

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