



# Facile “one-pot” synthesis of poly(methacrylic acid)-based hybrid monolith *via* thiol-ene click reaction for hydrophilic interaction chromatography



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## ABSTRACT

A novel sol-gel “one-pot” approach in tandem with a radical-mediated thiol-ene reaction for the synthesis of a methacrylic acid-based hybrid monolith was developed. The polymerization monomers, tetramethoxysilane (TMOS) and 3-mercaptopropyl trimethoxysilane (MPTS), were hydrolyzed in high-concentration methacrylic acid solution that also served as a hydrophilic functional monomer. The resulting solution was then mixed with initiator (2, 2'-azobis (2-methylpropionamide) dihydrochloride) and porogen (urea, polyethylene glycol 20,000) in a capillary column and polymerized in water bath. The column had a uniform porous structure and a good permeability. The evaluation of the monolith was performed by separation of small molecules including nucleosides, phenols, amides, bases and Triton X-100. The calibration curves for uridine, inosine, adenosine and cytidine were determined. All the calibration curves exhibited good linear regressions ( $R^2 \geq 0.995$ ) within the test ranges of 0.5–40  $\mu\text{g/mL}$  for four nucleosides. Additionally, atypical hydrophilic mechanism was proved by elution order from low to high according to polarity retention time increased with increases in the content of the organic solvent in the mobile phase. Further studies indicated that hydrogen bond and electrostatic interactions existed between the polar analytes and the stationary phase. This was the mechanism of retention. The excellent separation of the BSA digest showed good hydrophilicity of the column and indicated the potential in separation of complex biological samples.

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## 1. Introduction

Liquid chromatography (LC) is a powerful analytical technique in medicine, chemistry, life sciences, *etc.* [1–6]. Over the past few decades, reversed-phase liquid chromatography (RPLC) is the main separation mode and offers retention of most analytes. However, as an alternative to RPLC, hydrophilic interaction liquid chromatography (HILIC) has been playing an increasingly important role in the separation of polar compounds since it was first proposed by Alpert in 1990 [7–13]. The HILIC system consists of polar stationary phase such as silica [14,15] with cyano [16] or amino groups [15,17–19] and an aqueous organic solvent as the mobile phase.

The stationary phase is the core of chromatography. Its efficiency is always the concern of researchers. For high efficiency, traditional packing columns must have high pressure with small diameter support systems. However, the high pressure consumes much energy and requires special equipment. Compared with the traditional packing column, monolithic columns are a new type of stationary phase that could solve these problems *via* a rigid porous structure with a good permeability and high efficiency [20–22]. In all types of monolithic column reported, organic-silica hybrid monolithic columns, which combine the merits of both polymer-based and silica-based monolithic column [21,23], have attracted increased attention and have been widely applied in many fields [24–26]. Synthetic routes for these new organic-silica hybrid monolithic columns are mainly divided into two types: the use of functional organosilicas such as polyhedral oligomeric silsesquioxane (POSS-MA) as the monomer for polymerization and the combination of sol-gels with radical polymerization. Although these syntheses are becoming more mature, a lack of monomers

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with hydrophilicity and commercial availability of starting products limits the preparation of hydrophilic monolithic columns.

Click chemistry is widely applied in chemical synthesis because of its high efficiency and high selectivity [20,27–30]. Thiol-ene click chemistry is a novel kind of click chemistry that does not use metal catalysts. The free-radical polymerization occurs with vinyl and mercapto groups in functional monomers. The range of the monomers can be selected to enhance the final product and create diverse monolithic column surfaces. Currently, progress in the thiol-ene click reaction is focused on surface modification and radical-mediated “one-pot” methods.

Versus traditional derivatives using condensation of silanols, the thiol-ene click reaction has a wider range of use. Chen et al. [31] synthesized a monolithic enzyme microreactor by immobilizing the enzyme onto the hybrid monolithic column via a thiol-ene click reaction. The thiol-ene click approach provides a possible way to immobilize protein. On the basis of thiol-ene click chemistry, two other works were finished by same group by modifying the vinyl-based silica monolithic column with mercapto compounds, such as  $\beta$ -mercapto-1-propane-sulfonate/ $\beta$ -mercapto-1-propane-sulfonate [32] and 6-Mercapto-1-hexanol [33]. The column had enhanced hydrophilic ability and could be used as a reversed-phase stationary phase to separate small molecules. In these works, functional monomers are dispersed onto the surface of the monolithic column for surface modification. Nevertheless, in the radical-mediated “one-pot” method, all monomers are dispersed into the polymer solutions with more uniform and higher amounts of monomers distributed in the hybrid column. Yang et al. [34] prepared (3-sulfopropyl methacrylate potassium)-silica hybrid column with a “one-pot” approach based on the thiol-ene click reaction. Similarly, Chen et al. [35] prepared a monolith with 1, 2, 4-trivinylcyclohexane (TVCH) and pentaerythritol tetra(3-mercaptopropionate) (4SH) via photo-initiated thiol-ene click polymerization. The resulting monolithic column demonstrated excellent separation ability and high efficiency. Compared to the post modification strategy, the “one-pot” approach is more convenient and efficient way for the preparation of monolithic column.

For carboxyl-functionalized materials, Peng et al. [36] prepared a carboxyl-bonded stationary phase for hydrophilic interaction chromatography and the retention behaviors was enhanced with its charged state of the stationary phase. Zhao et al. [37] also prepared carboxyl-bonded silica materials through thiol-ene click chemistry to separate polar molecules with HILIC and weak cation-exchange mechanism. Horie et al. described a poly(acrylic acid) modified monolithic column prepared by using the post-modified strategy [38], the applicability of this column was demonstrated using nucleosides, carbohydrates, and peptides, indicating that the system has a capacity for highly efficient HILIC separation of polar molecules. However, these strategies mentioned above often involve cumbersome multistep sequences, which would limit its application. As far as we known, there is no known report of methacrylic acid as the monomer to prepare a hydrophilic hybrid monolithic column with the method of “one-pot” and radical-mediated thiol-ene click reactions.

In this study, a novel poly(methacrylic acid)-based hybrid monolithic column was fabricated via a sol-gel “one-pot” approach combined with a radical-mediated thiol-ene reaction using tetramethoxysilane (TMOS) and 3-mercaptopropyl trimethoxysilane (MPTS) as the inorganic monomers and methacrylic acid as the hydrophilic functional monomer (Fig. 1). The synthesis conditions were optimized in detail. The resulting monolithic column was characterized with optical microscopy, scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) and permeability testing. Toluene, benzyl alcohol, and thiourea were separated on the column at different linear velocities

to evaluate the column performance including hydrophilic chromatographic retention. The reproducibility and standard addition recovery test were also carefully examined with thiourea as a probe. The retention mechanism was comprehensively investigated with different compositions of organic solvent in the mobile phase and various additives into the mobile phase. The separation ability of the monolithic column was further examined via separation of small polar molecules including nucleosides, phenols, amides, bases and Triton X-100. A BSA digest was prepared to explore the potential application of the monolithic column in complex samples.

## 2. Experimental

### 2.1. Chemicals and materials

Tetramethoxysilane (TMOS, 99%), 3-mercaptopropyl trimethoxysilane (MPTS), 2'-azobis(2-methylpropionamide)dihydrochloride ( $V_{50}$ ), poly(ethylene glycol) (PEG,  $M_n = 20000$ ) and urea were purchased from J&K Chemical (Shanghai, China). Methacrylic acid (MAA) was purchased from Tianjin Kermel Chemical Plant (Tianjing, China). Bovine serum albumin (BSA), trypsin (1:250), dithiothreitol (DTT), iodoacetamide (IAA), toluene and other standard compounds were purchased from Sigma (St. Louis, MO, USA). Fused-silica capillaries with 100  $\mu\text{m}$  i.d., 150  $\mu\text{m}$  i.d. and 375  $\mu\text{m}$  o.d. were obtained from Reafine Chromatography Ltd. (Yongnian, Hebei, China). Acetonitrile (ACN, HPLC grade) was purchased from Merck (Darmstadt, Germany) and used for the preparation of mobile phases. The water was prepared with a Milli-Q system (Millipore Inc., Milford, MA). All the other chemical reagents were of analytical grade.

### 2.2. Preparation of the monolith

To enhance the integration of the monolith with the inner capillary wall, the inner wall of the fused-silica capillary must be treated with hydrochloric acid and sodium hydroxide. The fused-silica capillary was successively washed with 1.0 mol/L NaOH for 12 h, water for 30 min, 1.0 mol/L HCl for 12 h, and water for 30 min until the pH value of the outflow solution was neutral. This was followed with methanol for 30 min and nitrogen drying at room temperature for further use.

To fabricate the hybrid monolithic column, a solution of MAA (1 M, 1.3 mL), TMOS (400  $\mu\text{L}$ ) and MPTS (200  $\mu\text{L}$ ) was hydrolyzed at 0  $^\circ\text{C}$  for 4 h to form a homogeneous solution. Then, PEG (20,000 MW, 0.175 mg), urea (0.20 mg), and  $V_{50}$  (2 mg) were dissolved into the hydrolyzed mixture and ultra-sonicated at 0  $^\circ\text{C}$  for 10 min to mix and degass the solution. The homogeneous solution was manually introduced into the pretreated capillary with a syringe. Next, the capillary was held at 47  $^\circ\text{C}$  for 12 h for condensation and polymerization. Both ends were sealed with two pieces of rubber. Finally, the resulting monolithic column was rinsed with water and ACN to remove porogens and other residuals. Fig. 1 illustrates the principle of preparation of poly(methacrylic acid)-based hybrid monolithic column. The bulk monolith was polymerized in a 10 mL centrifugal tube similar to the optimized monolithic column. The blank bulk monolith was polymerized in a 10 mL centrifugal tube similar to the optimized monolithic column except for MAA. Here, the MAA solution was replaced with a HAc solution (0.01 mol/L).

### 2.3. Scanning electron microscopy

In addition to optical microscopy, SEM images were obtained with a JEOL JSM-6490 SEM (JEOL, Tokyo, Japan) to observe the microscopic morphology of the monolithic columns.

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