Journal of Chromatography A, 1271 (2013) 115–123

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Preparation of phenylboronic acid-silica hybrid monolithic column with one-pot approach for capillary liquid chromatography of biomolecules

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ARTICLE INFO

Article history:
Received 25 August 2012
Received in revised form 9 November 2012
Accepted 15 November 2012
Available online 21 November 2012

Keywords:
Capillary liquid chromatography
Organic–inorganic hybrid monolith
4-Vinylphenylboronic acid
Tryptic digest
Proteins

ABSTRACT

A phenylboronic acid-silica hybrid monolithic column for capillary liquid chromatography (cLC) was prepared through one-pot process by using 4-vinylphenylboronic acid (VPBA) and alkoxysilanes simultaneously. The effects of the molar ratio of tetramethyloxysilane/γ-methacryloxypropyltrimethoxysilane (TMOS/γ-MAPS), amount of VPBA, and the volume of diethylene glycol (DEG) on the morphologies, permeabilities and pore properties of the prepared VPBA-silica hybrid monolithic columns were studied in detail. A relatively uniform monolithic structure with high porosity was obtained with optimized ingredients. A series of cis-diol-containing compounds, alkylbenzenes, amides, and anilines were utilized to evaluate the retention behaviors of the VPBA-silica hybrid monolithic column. The result demonstrated that the prepared VPBA-silica hybrid monolithic column exhibited multiple interactions including hydrophobicity, hydrophilicity, as well as cation exchange apart from the expected affinity interaction. The run-to-run, column-to-column and batch-to-batch reproducibility of the VPBA-silica hybrid monolith were satisfactory with the relative standard deviations (RSDs) less than 1.63% ($n = 5$), 2.02% ($n = 3$) and 2.90% ($n = 5$), respectively, indicating the effectiveness and practicability of the proposed method. In addition, the VPBA-silica hybrid monolithic column was further applied to the separation of proteins and tryptic digest of bovine serum albumin (BSA), respectively. The successful applications suggested the potential of the VPBA-silica hybrid monolith in proteome analysis.

1. Introduction

In the post-genomic era, the growing demands for high-throughput protein analysis have stimulated the development of high efficient separation techniques and tools. Monolithic columns, as the fourth generation chromatography sorbents, have attracted considerable attention due to their unique properties such as low back pressure, fast mass transfer kinetics, high loading capacity and ease of preparation [1,2]. Based on the nature of the matrix chemistry, monolithic columns can be mainly classified into two types: the organic polymer-based and the inorganic silica-based monolithic columns. Organic polymer-based monoliths, including polyacrylate or polymethacrylate [3–5], polyacrylamide [6,7] and polystyrene [8,9], have good biocompatibility, excellent pH stability and great flexibility to tune the surface chemistry and therefore have been extensively developed over the past two decades. However, the swelling and shrinkage in organic solvents might lead to the change of pore structure of organic polymer-based monoliths, which in turn affects the mechanical stability of monolithic matrix and the retention reproducibility in some cases [10–12]. By contrast, inorganic silica-based monoliths demonstrate excellent solvent resistance and high mechanical stability [13–16]. Nevertheless, the surface functionalization of silica-based monolithic columns is time-consuming. Most critically, the synthetic process is difficult to control and often associated with the cracking and shrinking of silica skeleton during aging and drying [17,18].

As an alternative, the organic–inorganic hybrid monolithic columns that combine the advantages of silica-based with organic polymer-based monoliths, have recently gained great popularity in separation field [11,19–27]. The organic functional moieties can be incorporated into the inorganic silica monolithic matrix via either sol–gel [11,19,20] or one-pot process [21–27]. It is the sol–gel process that the organo-functionalized trialkoxysilanes (as the organic functional groups providers) are directly mixed with tetraalkoxysilanes. The alkoxy moieties undergo hydrolysis and condensation to form the hybrid silica matrix with the organic functionalities covalently incorporated. Although the method is simple, the choice of organo-functionalized trialkoxysilanes is rather limited. Different from the sol–gel

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http://dx.doi.org/10.1016/j.chroma.2012.11.038

process, one significant feature of one-pot approach is the simultaneous use of organic monomers and alkoxysilanes, in which polycondensation and polymerization were carried out in one pot by orderly changing reaction temperature. The use of the organic monomers in this one-pot process can circumvent the limitation of organic silanes for the organic–inorganic hybrid monolithic columns, opening a new way for preparation of various organic–inorganic hybrid monolithic columns with desirable organic functionalities. Moreover, the one-pot synthetic approach is extremely time-saving and high efficiency. Since the first report by Zou's group in 2009 [21], a variety of organic–inorganic hybrid monoliths with different organic functional monomers have been developed via one-pot approach [22–27], which has been successfully applied to the separation of nucleic acid bases and nucleosides, protein tryptic digests and enantiomers.

4-Vinylphenylboronic acid (VPBA), possessing a hydrophobic benzene ring and a hydrophilic/ionizable boronic acid group, was commonly used as functional probe for the recognition of cis-diol-containing molecules such as saccharides, RNA, nucleosides, glycoproteins and glycopeptides in the previous studies. The recognition principle was based on the reversible covalent complex formation/dissociation between boronic acids and cis diols in a basic/acidic aqueous media [28]. According to the recognition mechanism, Liu's group [26,29–31] and our group [27,32] have developed a series of polymer- and silica-based boronate affinity monoliths for the selective recognition and enrichment of nucleosides and glycoproteins. Apart from affinity interaction, secondary separation capacity between the analytes and the boronate functionalized monolith were also observed including reversed-phase, cation-exchange and hydrogen bonding interactions [26,30,33–37] and its secondary separation mechanism was clearly discussed in the recent review [38]. Recently, two different types of polymer-based monoliths, poly(glycidyl methacrylate-co-4-vinylphenylboronic acid-co-ethylene dimethacrylate) (poly(GMA-co-VPBA-co-EDMA)) and poly(4-vinylphenylboronic acid-co-pentaerythritol triacrylate) (poly(VPBA-co-PETA)) monoliths, have been designed by our group[39,40], both of which exhibited the typical mixed-mode chromatography toward test compounds. Most recently, Liu's group [26] reported one-pot synthesis of an organic-silica hybrid boronate affinity monolith using 3-acrylamidophenylboronic acid (AAPBA) as organic monomer. With this affinity monolith, recognition and separation of cis-diol containing compounds under neutral pH conditions can be obtained. Besides, hydrophilic interaction between nucleotides and AAPBA-silica hybrid monolith was also observed due to the introduction of hydrophilic AAPBA and silica matrix. Nevertheless, so far the advantages of organic-silica hybrid monolithic column have not been fully demonstrated yet. Further development is necessary to gain sound acknowledge and explore new applications of this type of monolithic column.

In this work, we described a facile method for one-pot synthesis of phenylboronic acid-silica hybrid monolithic columns by using the hydrolyzed tetramethyloxysilane (TMOS) and γ-methacryloxypropyltrimethoxysilane (γ-MAPS) as co-precursors and VPBA as functionalized organic monomer, respectively. The synthetic procedure was as simple as in situ polymerization of polymer-based monolith without any special handling. The influence of the ratio of TMOS to γ-MAPS, the amount of VPBA, and the content of porogenic solvent on the morphology, permeability and selectivity of the hybrid monoliths was investigated in detail. The applications of the newly designed hybrid separation media to separate a series of typical low-molecular-weight organic compounds, tryptic digests and proteins was also discussed in this work.

2. Experimental

2.1. Materials

VPBA and poly (ethylene glycol) (PEG, M_n = 10,000) were purchased from Alfa Aesar (Ward Hill, MA, USA). TMOS and γ-MAPS were products of Chemical Factory of Wuhan University (Wuhan, China). 2,2-Azobisisobutyronitrile (AIBN) was obtained from Tianjin Chemistry Reagent Factory (Tianjin, China) and recrystallized with methanol prior to use. All proteins (bovine serum albumin (BSA), bovine hemoglobin (BHb), cytochrome c (Cyt C), lysozyme (Lyz), myoglobin (Mb) and ribonuclease A (RNase A)) were obtained from Shanghai Lanji Co. Ltd. (Shanghai, China). Quinol and catechol were purchased from Sigma (St. Louis, MO, USA). Sequencing-grade modified trypsin (TPCK-trypsin) was from Promega (Madison, WI, USA). Alkylbenzenes, thiourea, anilines, HPLC-grade methanol (MeOH) and 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 μm o.d. × 75 μm i.d. were the products of Yongnian Optic Fiber Plant (Hebei, China).

2.2. Instruments

An organic–inorganic hybrid monolithic column with a total length of 50 cm (effective length 25 cm) was used unless otherwise stated. All chromatographic experiments were performed on a TriSep-2100 pressurized capillary electrochromatography (pCEC) instrument (Unimicro Technologies, Pleasanton, CA, USA) as described previously [39]. 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 μL 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 columns were carried out on a XL-30E scanning electron microscope (Philips, Netherlands). Surface area and pore size analysis were performed by Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) methods using physisorption analyzer (Micromeritics ASAP 2020 porosimeter, USA).

2.3. Preparation of the VPBA-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 [41]. The schematic preparation of VPBA-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), PEG 10,000 (540 mg), TMOS (1.8 mL), and γ-MAPS (0.5 mL) for 1 h at ice bath to form a homogeneous solution. Then, 25 mg of VPBA and 1 wt% AIBN dissolved with 80 μL diethylene glycol (DEG) were added into 0.5 mL of the resulting hydrolyzed mixture and then sonicated for 20 min. Afterward, the mixture was manually injected into the pretreated capillary to an appropriate length with a syringe. When both ends of the capillary were sealed with two pieces of rubbers, the capillary was incubated at 40 °C and 75 °C for 12 h, respectively. The obtained VPBA-silica hybrid monolithic column was flushed with MeOH to remove the residual monomers and porogens.