Journal of Chromatography A, 1358 (2014) 208–216

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Single-step approach for fabrication of vancomycin-bonded silica monolith as chiral stationary phase

Ming-Lung Hsieh [a], Lai-Kwan Chau [a,b,*], Yung-Son Hon [a,1]

[a] Department of Chemistry and Biochemistry, National Chung Cheng University, 168 University Road, Min-Hsiung, Chia-Yi 62102, Taiwan, ROC
[b] Center for Nano Bio-Detection and Advanced Institute of Manufacturing with High-tech Innovations, National Chung Cheng University, 168 University Road, Min-Hsiung, Chia-Yi 62102, Taiwan, ROC

ARTICLE INFO

Article history:
Received 17 March 2014
Received in revised form 28 June 2014
Accepted 2 July 2014
Available online 9 July 2014

Keywords:
Capillary electrochromatography
Vancomycin
Monolithic column
Sol–gel process
Chiral separation

ABSTRACT

A vancomycin-bonded silica monolithic column for capillary electrochromatography (CEC) was prepared by a single-step in situ sol–gel approach. This sol–gel process incorporates a synthetic sol–gel precursor which contains a macrocyclic antibiotic, vancomycin, to form a porous silica network inside a fused-silica capillary. To avoid degradation of vancomycin during the column fabrication, a mild step was adopted into the sol–gel process. The performance of the vancomycin chiral stationary phase was investigated by CEC in both the reversed-phase mode and the normal-phase mode. The vancomycin chiral stationary phase was optimized with respect to vancomycin loading in the reversed-phase mode for chiral separation of thalidomide enantiomers. The best efficiency and resolution values of 94 600 plates/m and 5.79, respectively, were achieved. The optimized column was further applied to chiral separation of alprenolol enantiomers. A plate height of less than 7 μm for the first eluted enantiomer of alprenolol was obtained in an aqueous mobile phase at a flow rate of 0.74 mm/s. Using enantiomers of seven β-blockers and some other basic enantiomers as test analytes, separation efficiencies of up to 148 100 plates/m in the reversed-phase mode and up to 138 100 plates/m in the normal-phase mode were achieved.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

The separation of racemic compounds is a very interesting topic of research in analytical chemistry, especially in the pharmaceutical field. Therefore, development of analytical chiral separation methods offering high efficiency and high resolution in a short time is highly desirable. In this perspective, capillary electrochromatography (CEC) is attractive as it combines the high efficiency of capillary electrophoresis (CE) and high selectivity of high-performance liquid chromatography (HPLC) [1–3]. Enantioselectivity in CEC can be achieved by the interactions with an appropriate chiral selector either bound or adsorbed to the capillary wall (open-tubular capillary) [4,5] or to a stationary phase (packed capillary or monolithic column) [6–10].

Monolithic stationary phases are increasingly considered as a viable alternative for columns packed with particles in HPLC and CEC because of their easy preparation, excellent properties and high performance [11–15]. Depending on the nature of the monolithic material, two major classes of monolithic columns can be identified: (1) organic polymer-based monoliths and (2) silica-based monoliths [11–15]. Organic polymer-based monoliths are created by a one-step polymerization of an organic monomer in the presence of a cross-linker, an initiator, and a porogen. A critical drawback associated with some organic polymer-based monolithic columns is its tendency to swell/shrink during exposure to the organic solvent in the running mobile phase. Such a structural change may reduce the mechanical stability and the permeability of the monolith. Silica-based monoliths, consisting of a silica skeleton and interconnecting macropores, generally are prepared using sol–gel technology. Inside the skeleton a large number of mesopores is present. The macropores can provide fast flow while the mesopores provide a large surface area which is necessary for a high sample loading. Additionally, the porous sol–gel network can offer high permeability, high efficiency, high mechanical strength, and good solvent resistance. The popularity of silica monoliths can be linked to the availability of different chemistries that can be used for surface modification and ligand attachment. As such, sol–gel

* Corresponding author at: Center for Nano Bio-Detection and Advanced Institute of Manufacturing with High-tech Innovations, National Chung Cheng University, 168 University Road, Min-Hsiung, Chia-Yi 62102, Taiwan, ROC.
Tel.: +886 5 2720411x66411; fax: +886 5 2721040.
E-mail address: chelkc@ccu.edu.tw (L.-K. Chau).
[1] Dedicated to the memory of Professor Yung-Son Hon (1955–2011).

http://dx.doi.org/10.1016/j.chroma.2014.07.003
0021-9673/© 2014 Elsevier B.V. All rights reserved.

processing presents an attractive inorganic alternative for preparation of silica-based monoliths [16].

Silica monoliths fabricated by sol–gel processing often incorporate the use of porogen, say, poly(ethylene glycol) (PEG), to manipulate phase separation and thus control macropore size and volume [11]. Monoliths thus formed generally possess high through-pore-to-skeleton size ratios to provide good column permeability and separation efficiency. The chiral selectors utilized for the preparation of silica-based monoliths ranged from cyclodextrin and its derivatives, macrocyclic antibiotics, chiral ion-exchangers, proteins, ligand exchange-chiral stationary phases, cellulose derivatives to molecular chiral imprinted polymer [17–25]. Up to now, the immobilization of a chiral selector in most of these CEC columns was based on in situ encapsulation or physical adsorption or post-modification of silica monoliths. In 2000, Hayes and Malik used a C18-silicon alkoxide to prepare monolithic octadecylated silica columns in a single step for CEC [26]. Our previous study used a sol–gel precursor with a quaternary ammonium functionality to prepare monolithic columns in a single step for anion-exchange CEC [27]. Recently, a single-step approach to prepare monolithic columns by sol–gel processing of an organofunctional silicon alkoxide precursor that contains a macrocyclic moiety, cyclodextrin, was also described by our group [28].

The powerful enantioseparation capability of glycopeptide antibiotics was first introduced by Armstrong and coworkers as chiral selectors in HPLC and later on widely applied to CE system for separation of enantiomers [29–32]. Macrocyclic glycopeptides are efficient chiral selectors for several reasons: (1) they contain ionizable functional groups, which can be either acidic or basic depending on pH; (2) they have multiple stereogenic centers; (3) they process numerous functional groups contributive to stereoselectivity; and (4) they contain both hydrophobic and hydrophilic group. Hence, the chiral separation mechanism to form transient noncovalent diastereomeric complexes with glycopeptides antibiotic is based on electrostatic interactions as well as secondary interactions such as hydrophobic, hydrogen bonds, dipole-dipole, π–π interactions, and steric repulsion. In which, vancomycin and teicoplanin are the most frequently used and have successfully applied to HPLC and also to CEC for chiral separation of compounds of pharmaceutical interest using packed stationary phases in the reversed-phase and the normal-phase modes [8,33–37].

So far, many vancomycin stationary phases (such as commercial Chirobiotic V™ and LiChrospher® diol silica modified with vancomycin) have been applied to CEC in the form of packed columns. Nevertheless, a comprehensive survey of enantioseparations by CEC using vancomycin or norvancomycin monolithic column shows only few publications [9,20,38–40]. Maruska and coworkers used an organic polymeric continuous-bed and post-modified with vancomycin as a chiral stationary phase (CSP) [9,38]. Dong and coworkers used a silica monolith and post-modified with vancomycin [20]. Ding and coworkers used a silica monolith and post-modified with norvancomycin [39,40]. Enantioseparations by HPLC using vancomycin monolithic column has also been reported by Pittler and Schmid using dynamic coating with a vancomycin derivative [41].

Although post-modified monolithic column has found applications in various fields, the elimination of such complicated tasks of chemical treatment is desirable. Here, we describe a new and easy-to-prepared method to fabricate a well-controlled functional 3D skeletal silica monolith based on a single-step in situ sol–gel process. The vancomycin CSP precursor for sol–gel processing was synthesized via addition reaction by covalently attaching triethoxysilyl group to the vancomycin moiety. Copolymerization of the vancomycin CSP precursor with tetramethoxysilane (TMOS) under acid-catalyzed sol–gel reaction results in a functionalized monolithic column. During the sol–gel process, proper addition of PEG will cause phase separation [11]. Upon removal of PEG, a porous monolithic stationary phase for CEC separation is ready. Thus, the time and labor associated with column fabrication are reduced.

To the best of our knowledge, we are the first to prepare vancomycin-bonded silica monolithic columns by a single-step in situ sol–gel process. The capillaries thus formed were tested for the CEC chiral separation of a number of racemates of pharmaceutical interest. In particular, chiral separation of the enantiomers of β-blockers has been successfully achieved with good efficiency and resolution in both the reversed-phase mode and the normal-phase mode.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were of highest available purity and used directly without any further pretreatment. Tetramethoxysilane (TMOS) was obtained from Acros Organics (USA). β-Blockers (acebutolol, alprenolol, atenolol, labetalol, metoprolol, pindolol, propranolol), bromacil, bupivacaine, devrinol, thalidomide, and warfarin were obtained from Sigma–Aldrich (USA). Poly(ethylene glycol) (PEG) with an average molecular mass of 10 000, glacial acetic acid (HOAc), and 3-isocyanatopropyltriethoxysilane (ICNPTES) were obtained from Fluka Chemika (USA). *N,N*-Dimethylformamide (DMF), acetone, acetonitrile (ACN), ethanol, diethyl ether, and methanol (MeOH) were purchased from TEDIA Company (USA). Triethylamine (TEA) and Coumachlor were obtained from Riedel-De Haën (German). Vancomycin was purchased from Duchefa Biochemie (Nederlands). Triethylammonium acetate (TEAA) buffer was prepared by adjusting 0.1% or 1% solution of TEA with HOAc to the appropriate pH. Ammonium acetate solution was prepared by dissolving 10 mmol of acetic acid in water, adding ammonium hydroxide up to the desired pH value, and diluting to the final volume of 100 mL with water. The pH values of the running electrolytes were measured by an Orion 420A pH meter (USA). All aqueous solutions were prepared with water that had been purified by a Milli-Q water purification system (Millipore) with a specific resistance of 18.2 MΩ cm. Aqueous mobile phases were prepared by adding the desired volume of the HPLC grade organic solvents to the pH controlled buffer solutions. Before use, all solutions were filtered through a 0.22 μm membrane (Corning) and degassed by vacuum and sonication. Polar organic mobile phases were prepared by adding a desired ratio of TEA and HOAc to a mixture of MeOH and ACN. Analyte stock solutions (4.0 mg/mL) were dissolved into the HPLC grade MeOH or ACN and stored at 4 °C. The racemic samples for CEC experiments were daily diluted to the desired concentrations with an aqueous buffer or MeOH.

2.2. Synthesis of vancomycin CSP precursor

To a stirred solution of vancomycin (60 mg, 0.04 mmol) in 0.5 mL dry DMF, ICNPTES (30 μL, 0.12 mmol) and TEA (12 μL, 0.07 mmol) were added. The resulting solution was stirred at room temperature under nitrogen for 24 h. After adding diethyl ether (6 mL) and stirring for another 5 min, the precipitate collected by centrifugation was rinsed by diethyl ether. The purified precipitate was dried under vacuum to afford the title compound as a light yellow solid and identified by a LTQ ion trap mass spectrometer (Thermo Electron Corp., CA). The product was then used as the CSP precursor for further sol–gel processing.

Download English Version:

https://daneshyari.com/en/article/7612763

Download Persian Version:

https://daneshyari.com/article/7612763

Daneshyari.com