Microchemical Journal 120 (2015) 18–25

Contents lists available at ScienceDirect

Microchemical Journal

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

Maximizing the practical value and investigating the retention characteristics of a remodified first-generation monolith

Arianne Soliven [a,b,*], Gary R. Dennis [a], R. Andrew Shalliker [a]

[a] Australian Centre for Research on Separation Science (ACROSS), School of Science and Health, University of Western Sydney, Parramatta, NSW, Australia
[b] Department of Natural Products and Pharmacognosy, Faculty of Chemistry, Universidad de la República, Montevideo, Uruguay

ARTICLE INFO

Article history:
Received 27 November 2014
Received in revised form 20 December 2014
Accepted 20 December 2014
Available online 29 December 2014

Keywords:
Monolith
Silica monolith
Modified monolith
Selectivity
First-generation monolith
Phenyl monolith
Cyano monolith
HPLC

ABSTRACT

This study illustrates a procedure for producing different high-performance liquid chromatography (HPLC) stationary phases, on demand, for silica monoliths. Two commercial first-generation analytical scale bare silica monoliths were subjected to a "remodification" process that involved the following: (1) coating of the silica through an *in situ* silylation procedure to bond ligands of choice to the surface, (2) periodic chromatographic characterization of the coated surface over 2,000 column volumes, and (3) removing the bonded ligands using a 0.1 M hydrochloric (HCl) acid wash, to regenerate the initial silica surface.
This remodification protocol was repeated for three cycles to fabricate monoliths with selected functionalities. To test reproducibility, this study was conducted on two different commercial silica monoliths subjected to this treatment involving synthetic reactions with organo-silanes and subsequent stripping with acid washes. Both monoliths were functionalized with three different coatings, without significant degradation of the initial columns' silica infrastructure and excellent stability (tested up to 2,000 column volumes). One of the monoliths had measured theoretical plates per meter (*N*/m) of 57,000 *N*/m for the original silica surface, 105,000 *N*/m for the first cyano coating, and 60,000 *N*/m for the third phenyl coating. The stationary phases prepared by remodification possessed similar selectivities to those synthesized from new silica monoliths. The results of the Tanaka test shows evidence of residual ligands of previous coatings. However, the methylene and phenyl selectivity proved to display the same characteristics as other modified monoliths of the same functionality, not subjected to the remodified process.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

The high-speed separation advantages of first-generation monolithic columns have been well discussed in the literature [5–10], yet their widespread use within the chromatographic industry is limited. One possible reason is due to the limited selectivities that are commercially available (native silica, C8, and C18) [6,11]. A more extensive range of stationary phases is thus required. The preparation of silica monoliths is extremely difficult, and it has been stated that one research group is successful at producing high quality rod encapsulated silica based monoliths for chromatographic purposes, with strong patents protecting the process [6]. Due to the difficulties faced by the preparation process, the selectivity limitation may be overcome by employing the *in situ* modification method to functionalize the monolith [1–4]. Most of the *in situ* methods have been directed towards micro-scale chromatography, a few developed for analytical-sized columns [12–16], all of which have been comprehensively reviewed [6,8]. Recently, our group has developed a simple and successful *in situ* procedure to modify commercial analytical scale monoliths [1–4].

Bonding a C18 selectivity to the silica framework was described in detail in the first body of work and was compared to a commercially available C18 monolith [2]. The preparation of non-commercially available stationary phases (cyano, phenyl, and mixed-mode selectivities) and the chromatographic characterization of the modified monolith's performance have also been studied [1–4]. The effects of endcapping using the *in situ* method improved efficiency and decreased the residual silanol groups by up to 35%, while also maintaining the phenyl selectivity behavior, although pre and post endcapping entailed differences in methylene selectivity [3]. In the present study, the new stationary phase is prepared, then stripped from the silica surface using acid, to regenerate the initial silica surface. This was repeated for several cycles. The purpose of this exercise of repeating *in situ* derivatization and coating removal was to examine if the purchase of one native silica monolith could provide a variety of different stationary phase selectivities when required by the analyst, and with relative ease.

Each coated surface of the "remodified" monolith was tested chromatographically in the exact same fashion with the previous studies [1–4], the retention characterization tests were carefully selected to have a consumed amount of mobile phase of <2,000 column volumes.

* Corresponding author at: Australian Centre for Research on Separation Science (ACROSS), School of Science and Health, University of Western Sydney, Parramatta, NSW, Australia. Tel.: +61 2 9685 9918; fax: +61 2 9685 9915.
E-mail addresses: a.soliven@uws.edu.au, asoliven@fq.edu.uy (A. Soliven).

http://dx.doi.org/10.1016/j.microc.2014.12.009
0026-265X/© 2014 Elsevier B.V. All rights reserved.

Testing was completed before proceeding with an acid wash to regenerate the original bare silica skeleton. The retention characterization tests included column efficiency height equivalent to a theoretical plate (HETP) curves to determine the performance as a function of linear velocity [17], longevity studies (stability of the surface), selectivity studies using Snyder and Dolan's linear solvent strength model (*LSS* model) [18], and the Tanaka test [19].

The selectivity study utilized changes in retention as a function of the solvent composition for homologues, described by Eq. (1):

$$\log k = \log k_w - S\bullet\phi \quad (1)$$

where k_w is the retention in pure water, ϕ is the organic modifier component of the mobile phase, and S is the slope of the linear relationships of ϕ and log k. The *LSS* model has proven critical for gradient peak capacity optimization procedures [20] and studies investigating the methylene and phenyl selectivity behavior using a set of alkylbenzenes and linear polycyclic aromatic hydrocarbons (PAHs), through plots of S vs. n (where n represents the nth member of the family of analytes) [1–4,21–24]. Discontinuity of S vs. n means convergence of the *LSS* relationships, hence complicating the strategic optimization of separations for the column tested [22,23]. Therefore, these selectivity plots provide a good approach to characterize the ability of a stationary phase to separate closely related species.

A fast and effective means to characterize a column for various properties (hydrophobicity, steric selectivity, hydrogen bonding capacity, amount of alkyl chains, and ion exchange sites under acidic and basic conditions) can be achieved by using the Tanaka test [19]. The Tanaka test was validated previously against a laborious 101 solute (differing in shape, size, and functionality) on five different reversed phase columns [25]. This serves as a rapid test in comparison to the Galushko model [26] and other tests [27–31].

The investigation of the retention characteristics of a remodified first-generation monolith is important to demonstrate the practical value of including this column format in a separation scientist's artillery of columns. In most cases, a column collection would vary in length, format, and selectivity. The first-generation monolith tailor made to specific lengths, to match the demands of two-dimensional HPLC, has been demonstrated in a study by Stevenson and Shalliker [32]. A "remodified" monolith offers two advantages: (1) monoliths other than C18, or C8 can be employed, as there is no limit to the functionality that can be derivatized, and (2) the cost of acquiring a range of different selectivities in monolithic stationary phases is reduced, as the same scaffold can be employed numerous times.

2. Experimental

2.1. Chemicals

HPLC-grade methanol, isopropanol, and heptane were obtained from Merck Pty. Ltd. (Kilsyth, Victoria, Australia). HPLC-grade tetrahydrofuran was obtained from LabScan Analytical Sciences distributed by LOMB Scientific (AUST) Pty. Ltd. (Taren Point, NSW, Australia). Test solutes (acetone, *p*-cresol, benzene, anisole, toluene and phenetole, ethylbenzene, propylbenzene, butylbenzene, hexylbenzene, naphthalene, anthracene, 2,3-benzanthracene, pentacene, triphenylene, *o*-terphenyl, caffeine, phenol, amylbenzene, and benzylamine) were obtained from the Aldrich Chemical Company, Inc. (Sigma-Aldrich Chemical Company Inc., Castle Hill, NSW, Australia). Heptane was dried by reflux and then distilled from sodium before use. Octadecyldimethylchlorosilane, trimethylchlorosilane, (3-cyanopropyl)dimethylchlorosilane, and (3-phenylpropyl)dimethylchlorosilane were obtained from Gelest (USA). All materials were used as received. All mobile phases were prepared gravimetrically (±0.01 g), delivered through a single line and used without further filtration.

2.2. Equipment

Chromatographic tests were performed on a Shimadzu LC system (Shimadzu Scientific Instruments, Rydalmere, NSW, Australia), incorporating an LC-10AT*VP* pumping system, SIL-10AD*VP* auto injector, DGU-14A online degasser, SPD-M10A*VP* diode array detector (set at 254 nm), and Shimadzu Class-*VP* version 6.14 software on a Pentium III 700 MHz processor. In addition to the online degasser, mobile phases were periodically sparged with helium. The temperature of the column was thermostated at 30 °C using an HPLC column heater (Thermasphere TS-130) from Phenomenex Pty Ltd (Lane Cove, NSW, Australia).

Two Onyx silica monoliths (100 × 4.6 mm) were purchased from Phenomenex Pty. Ltd. (Lane Cove, NSW, Australia).

2.3. Ligand bonding procedure (in situ modification method)

The method used to modify the bare monolith was similar to the method reported previously [2]. Prior to surface modification, dried heptane (50 mL) was pumped through the monolith. Dependent on the choice of coating: (3-cyanopropyl)dimethylchlorosilane (CN coating), octadecyldimethylchlorosilane (C18 coating), or (3-phenylpropyl) dimethylchlorosilane (phenyl coating), a 1% v/v solution of the selected silane, in dried heptane was used, called the "ligand bonding silane" solution. A 1% v/v solution of trimethylchlorosilane was used as the "endcapping silane" solution.

The ligand bonding silane solution was pumped through the monolith (5 column volumes) at 30-min intervals (at flow rates of up to 4 mL/min), until 200 mL of the ligand bonding silane solution had been passed through the monolith. This step was repeated in the reverse column flow direction (opposite flow direction indicated by the column manufacturer).

Once the selectivity of choice was coated to the surface, the endcapping silane solution was passed through the monolith. At 30-min intervals—5 column volumes was eluted at 4 mL/min, until a total of 100 mL was passed through (in the forward direction only).

The ligand bonding and endcapping silane solutions were pumped through the monolith using a Waters 501 HPLC pump thermostated at 80 °C using an HPLC column heater (Thermasphere TS-130) from Phenomenex.

After completion of the silylation, the monolith was washed at room temperature with separate portions of 100% heptane (50 mL), isopropanol (30 mL), and methanol (30 mL) using flow rates of up to 4 mL/min.

2.4. Ligand removal procedure (silica surface regeneration)

After completing the chromatographic performance tests on the bonded monolith phase, the ligands were removed to regenerate the initial silica surface. The ligand coating was removed using an acid wash (~1200 mL of 0.1 M HCl) pumped through at a flow rate between 3 and 5 mL/min. The column was then washed with methanol/water (10/90) to neutral pH.

2.5. Stability

The stability of the column, i.e., silica bed quality and surface coating, was tested using a standard test mix of substituted aromatics (acetone, benzene, anisole, toluene, and phenetole) dissolved in methanol/water (30/70 for the phenyl phase and 40/60 for the phenyl endcapped phase) and made up to concentrations between 3 and 52 mmol/L. Column performance was tested initially and again after the completion of a chromatography study (methanol/water). The log of the retention factors (log k) was calculated for each test solute and compared throughout the entire duration of the experimental work to ensure the integrity of the stationary phase. These longevity tests sought only

Download English Version:

https://daneshyari.com/en/article/1227706

Download Persian Version:

https://daneshyari.com/article/1227706

Daneshyari.com