Journal of Chromatography A, 1217 (2010) 8212–8221

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Hypercrosslinking: New approach to porous polymer monolithic capillary columns with large surface area for the highly efficient separation of small molecules

Jiri Urban[a], Frantisek Svec[b], Jean M.J. Fréchet[a,b,*]

[a] Department of Chemistry, University of California, Berkeley, CA 94720, USA
[b] The Molecular Foundry, E.O. Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

ARTICLE INFO

Article history:
Received 13 September 2010
Received in revised form 22 October 2010
Accepted 25 October 2010
Available online 31 October 2010

Keywords:
Hypercrosslinked polymer monolith
Poly(styrene-*co*-vinylbenzyl chloride-*co*-divinylbenzene)
Reversed phase chromatography
Size-exclusion chromatography
Small molecules

ABSTRACT

Monolithic polymers with an unprecedented surface area of over 600 m^2/g have been prepared from a poly(styrene-*co*-vinylbenzyl chloride-*co*-divinylbenzene) precursor monolith that was swollen in 1,2-dichloroethane and hypercrosslinked via Friedel-Crafts reaction catalyzed by ferric chloride. Both the composition of the reaction mixture used for the preparation of the precursor monolith and the conditions of the hypercrosslinking reaction have been varied using mathematical design of experiments and the optimized system validated. Hypercrosslinked monolithic capillary columns contain an array of small pores that make the column ideally suited for the high efficiency isocratic separations of small molecules such as uracil and alkylbenzenes with column efficiencies reproducibly exceeding 80,000 plates/m for retained compounds. The separation process could be accelerated while also improving peak shape through the use of higher temperatures and a ternary mobile phase consisting of acetonitrile, tetrahydrofuran, and water. As a result, seven compounds were well separated in less than 2 min. These columns also facilitate separations of peptide mixtures such as a tryptic digest of cytochrome c using a gradient elution mode which affords a sequence coverage of 93%. A 65 cm long hypercrosslinked capillary column used in size exclusion mode with tetrahydrofuran as the mobile phase afforded almost baseline separation of toluene and five polystyrene standards.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Monolithic columns with excellent permeability to flow emerged in the early 1990s and enabled rapid chromatographic separations at high flow velocities [1–4]. Theoretical models developed later by Liapis, Guiochon, Desmet, and others assigned their enhanced performance to low resistance to mass transfer enabled by morphological features of their internal structure [5–9]. Detailed overview of the characteristics and applications of polymer-based monolith is presented in many review articles [10–22]. Typical monolithic columns prepared from porous polymers [3] comprise aggregated nonporous microglobules and exhibit modest surface areas in the range of tens of m^2/g. Since these monolithic columns completely lack mesopores, they are almost ideally suited for the fast separation of molecules such as proteins [23], nucleic acids [24], and synthetic polymers [25] using the gradient elution mode. While the small surface area of current polymer monoliths provides distinct advantages in the separations of large molecules for which diffusional mass transport is slow, it does not provide the desirable high number of interaction sites required for the separation of small molecules in isocratic mode. For example, in our early experiments, we found that monolithic poly(styrene-*co*-divinylbenzene) column separated alkylbenzenes with an efficiency of only 18,000 plates/m [26]. Several recent attempts to optimize the porous properties of methacrylate polymer monoliths have led to columns affording 35,000–50,000 plates/m for benzene as the retained analyte [27–31]. In addition, several new approaches have also been explored including the polymerization of a crosslinking monomer [32–35], the termination of the polymerization reaction at an early stage [36–38], and the use of polymerization at high temperatures [39–41]. In spite of all of these efforts, it has always proven difficult to prepare polymer monoliths possessing both large through pores and a multiplicity of small pores in a single step and alternative approaches to do so are highly desirable.

Several decades ago, Davankov prepared large surface area materials from preformed polymer precursors in a technique termed "hypercrosslinking" [42–45]. The original implementation involved linear polystyrene crosslinked via a Friedel-Crafts alkylation with bis-electrophiles to afford materials containing mostly

* Corresponding author at: Department of Chemistry, University of California, Berkeley, CA 94720, USA. Tel.: +1 510 643 3077; fax: +1 510 643 3079.
E-mail address: frechet1@gmail.com (J.M.J. Fréchet).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.10.100

small pores [42,46]. Davankov later extended hypercrosslinking to previously crosslinked polystyrene copolymers [44]. Other groups carried out hypercrosslinking using porous poly(vinylbenzyl chloride-*co*-styrene-*co*-divinylbenzene) resins [47–50]. For example, Sherrington and coworkers have prepared polymer beads with a bimodal pore size distribution and surface areas as high as 1055 m^2/g [48]. Similarly, we hypercrosslinked macroporous poly(vinylbenzyl chloride-*co*-divinylbenzene) to obtain beads with a surface area exceeding 2000 m^2/g [51,52]. Small diameter hypercrosslinked beds have also been used as stationary phase in HPLC [53–55].

Recently, we have demonstrated for first time the preparation of hypercrosslinked porous polymer monoliths exhibiting a large surface area and their use in capillary columns for the fast and efficient separation of small molecules as well as for rapid size-exclusion chromatography [56].

In this report we focus on the optimization of reaction conditions for the preparation of highly performing hypercrosslinked monolithic capillary columns. We also explore the factors that affect the formation of network of small pores within the monolith for enhanced column efficiencies.

2. Experimental part

2.1. Materials

Styrene (99%), vinylbenzyl chloride (mixture of 3- and 4-isomers, 97%), divinylbenzene (80%, technical grade), 2,2′-azobisisobutyronitrile (98%), acetonitrile (HPLC grade), uracil, benzene, ethylbenzene, amylbenzene, ribonuclease A, cytochrome c, myoglobin, α-chymotrypsinogen A and albumin were all obtained from Sigma–Aldrich (St. Louis, MO, USA). Propylbenzene and butylbenzene were purchased from Matheson Coleman & Bell (Los Angeles, CA, USA), toluene from EMD Chemicals (Gibbstown, NJ, USA). The monomers styrene, vinylbenzyl chloride, and divinylbenzene were purified by passage through a bed of basic alumina to remove the inhibitors. Ferric chloride was purchased from Fisher (New Jersey, NJ, USA). Polystyrene standards with molar masses ranging from 580 to 1,870,000 were obtained from Viscotek (Houston, TX, USA). Polyimide-coated 100 μm I.D. fused silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA).

2.2. Preparation of capillary columns

The fused-silica capillaries were rinsed with acetone, water, 200 mmol/L sodium hydroxide, water, 200 mmol/L HCl, and ethanol. Then, a 20% (v/v) solution of 3-(trimethoxysilyl)propyl methacrylate in ethanol with an apparent pH value of 5 adjusted using acetic acid, was pumped through the capillary for 1 h using a syringe pump. After this vinylization procedure, the capillaries were rinsed with ethanol, and dried with a stream of nitrogen.

Generic monoliths were prepared in capillaries using *in situ* polymerization of mixtures of styrene, vinylbenzyl chloride, and divinylbenzene dissolved in binary porogen solvent containing toluene and 1-dodecanol. 2,2′-Azobisisobutyronitrile (AIBN) (1%, w/w, with respect to monomers) was used as the initiator. The polymerization mixtures were purged with nitrogen for 10 min and filled in the vinylized capillaries using a syringe. Both ends of the capillary were sealed with rubber stoppers and the capillary was placed in a water bath. Polymerization was carried out at 70 °C for 20 h. Both ends of the capillary were then cut to adjust its lengths and the monolithic column was washed with acetonitrile. Simultaneously, excess of the polymerization mixture was polymerized in a glass vial under the same conditions. The vial containing the monolith formed from the bulk polymerization mixture was carefully crushed, the polymer cut into small pieces, Soxhlet extracted with methanol for 12 h to remove any soluble compounds, and vacuum dried at 60 °C overnight. This polymer was used for the porosimetric measurements.

2.3. Hypercrosslinking

The hypercrosslinking reaction in batch was carried out using 1.70 g monolithic material pre-swollen in 20 mL of 1,2-dichloroethane for 2 h. The Lewis acid catalyst $FeCl_3$ (1 g) was then added to the slurry cooled in an ice bath. Once the catalyst was homogeneously dispersed, the mixture was allowed to come to the room temperature. The hypercrosslinking reaction was then allowed to proceed at 80 °C for 24 h, unless mentioned otherwise. The resulting polymer was separated and washed with methanol, then 0.5 mol/L HCl in acetone, and then again with methanol followed by drying *in vacuo*. Hypercrosslinking of monoliths in capillary columns followed a similar procedure. The columns were flushed with 1,2-dichloroethane at a flow rate of 0.25 μL/min for 2 h. The filtered solution of 1 g of $FeCl_3$ in 20 mL of 1,2-dichloroethane was pumped through the columns at a flow rate of 0.25 μL/min for 2 h and the columns were held in an ice bath for 1 h. Unless otherwise mentioned, the reaction was carried out at 80 °C for 2 h. The hypercrosslinking columns were then washed with water overnight and tested.

2.4. Instrumentation

A Dionex Ultimate 3000 HPLC system (Sunnyvale, CA, USA) equipped with a 3-nL UV detection cell and an external micro-valve injector with a 4-nL inner sampling loop (Valco, Houston, USA) was used for the chromatographic experiments. Typically, isocratic reversed-phase chromatography was carried out using a mixture of 80% acetonitrile and 20% water as the mobile phase. Solutions of 0.1% formic acid in acetonitrile and water, respectively, were used as the mobile phases for elution in the gradient mode. Tetrahydrofuran at a flow rate of 0.5 μL/min and polystyrene standards were used for size-exclusion chromatography.

Nitrogen adsorption/desorption isotherms were measured using a Micromeritics ASAP 2010 surface area and porosimetry analyzer (Norcross, GA) and used for the calculation of surface areas.

2.5. Separation of tryptic digest

Cytochrome c (2.5 mg/mL) was diluted with a 100 mmol/L ammonium bicarbonate solution, trypsin added at a substrate-to-enzyme ratio of 50:1 (w/w) and the solution was incubated at 37 °C for 20 h. The proteolysis was terminated by lowering the pH via addition of formic acid to the solution. Chromatographic experiments with mass spectrometric (MS) detection were performed using a liquid chromatography system consisting of an Agilent 1200 Series capillary pump and external Valco injector with 4 nL injection loop. Linear gradient of 5–40% acetonitrile with 0.1% formic acid in 0.1% aqueous formic acid was used at a flow rate of 0.5 μL/min. MS detection was carried out using a micrOTOF-Q (Bruker Daltonics, Fremont, CA, USA) equipped with nanospray interface in positive ion mode with a m/z range of 400–1500.

2.6. Chromatographic characterization

The effect of linear velocity of the mobile phase, u, on efficiency of hypercrosslinked monolithic capillary columns defined as height equivalent to theoretical plate, HETP, was determined from plots described by van Deemter equation [57].

Download English Version:

https://daneshyari.com/en/article/1203448

Download Persian Version:

https://daneshyari.com/article/1203448

Daneshyari.com