Journal of Chromatography A, 1396 (2015) 54–61

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Pore volume accessibility of particulate and monolithic stationary phases

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ARTICLE INFO

Article history:
Received 13 January 2015
Received in revised form 26 March 2015
Accepted 27 March 2015
Available online 4 April 2015

Keywords:
Accessibility
Efficiency
Inverse size-exclusion chromatography
Polymer monoliths
Pore volume

ABSTRACT

A chromatographic characterization of pore volume accessibility for both particulate and monolithic stationary phases is presented. Size-exclusion calibration curves have been used to determine the pore volume fraction that is accessible for six alkylbenzenes and twelve polystyrene standards in tetrahydrofuran as the mobile phase. Accessible porosity has been then correlated with the size of the pores from which individual compounds are just excluded.

I have determined pore volume accessibility of commercially available columns packed with fully and superficially porous particles, as well as with silica-based monolithic stationary phase. I also have investigated pore accessibility of polymer-based monolithic stationary phases. Suggested protocol is used to characterize pore formation at the early stage of the polymerization, to evaluate an extent of hypercrosslinking during modification of pore surface, and to characterize the pore accessibility of monolithic stationary phases hypercrosslinked after an early termination of polymerization reaction. Pore volume accessibility was also correlated to column efficiency of both particulate and monolithic stationary phases.

1. Introduction

Besides surface chemistry, controlling the stationary phase selectivity, the porous and hydrodynamic characteristics of the stationary phase are the most important factors affecting a separation performance in liquid chromatography-based separations [1]. Currently, the family of the stationary phase's materials includes totally porous, superficially porous and non-porous particles as well as a silica-based and organic polymer-based monolithic stationary phases [2].

In contrast to columns packed with particles, monolithic separation media consist of a single piece of a highly porous material. The monolithic bed forms a highly interconnected network of relatively large-size channels (macropores) allowing mobile phase to flow through the bed. In contrary, the proportion of inner pores (micropores and mesopores) is much lower than that for particulate column packings [3–5]. Wide variety of monomers can be used for the preparation of polymer monoliths [5] and several preparation protocols were introduced to improve efficiency in the separation of small molecules [6].

The basic parameters used for a physical description of the stationary phase include pore size and pore size distributions, specific surface area and (in case of particulate stationary phases) particle size and particle size distribution. Various methods have been used to determine the porous properties of chromatographic stationary phases. The most-common methods include nitrogen adsorption [7] and mercury-intrusion porosimetry [8]. Besides the classical techniques, the internal structure of polymer and silica monolithic stationary phases has also been characterized by an electron microscopy technique [9,10]. The most commonly applied scanning electron microscopy and transmission electron microscopy provide only two-dimensional information about the structural properties [11]. The three-dimensional information can be reconstructed by an application of focused ion beam–scanning electron microscopy or serial block-face scanning electron microscopy [10,12]. Although providing very important structural information and morphology characteristics, these methods require expensive instrumentation and laborious workflow.

A major question in characterizing the porous properties of monolithic beds is the extent to which the porous properties of the "dry" monoliths are indicative of the chromatographic performance under "wet" (swollen or solvated) conditions. While the morphological characteristics can be constant in the case of silica-based monoliths, the porous properties of polymer monoliths are affected significantly by the swelling solvent [13,14]. We found that

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http://dx.doi.org/10.1016/j.chroma.2015.03.078
0021-9673/

substantial amount of small pores is available only when a polymer-based monolithic stationary phases is solvated by a mobile phase [14] and can be attributed to so called "gel porosity" that has been predicted [15] and recently thoroughly studied by Nischang [16–19]. The extent of a gel porosity is controlled by the crosslink density distribution. The lower the degree of crosslinking, the larger amount of the polymers porous structure consists of gel porosity [20]. Moreover, the gel porosity can also be modulated by the composition of the mobile phase [13,21].

Hence, a characterization of polymer stationary phases should be performed utilizing methods that determine porous properties in a presence of a swelling solvent, preferably as close to the applied mobile phase as possible. For example, Chambers et al. used scanning ion conductance microscopy to characterize successful incorporation of carbon nanotubes to a porous monolithic material [22]. Laher et al. characterized commercially available polymer monoliths by using a confocal Raman spectroscopy imaging that can be advantageously used in both dry and solvated states [23]. Recently, we have used remote detection LC–NMR experiments to characterize mobile phase flow profile in hypercrosslinked polymer-based stationary phases [24]. We found nearly plug-like profile illustrating that monolithic pore structure evenly distributes the mobile phase across the column and acts as a frit. Although not a real porosimetry technique, this method provides description of an overall (monolithic) stationary phase homogeneity necessary for improvements of tailored stationary phases.

Inverse size-exclusion chromatography (ISEC) is only one chromatographic technique providing porous properties of stationary phases. ISEC was introduced by Halász [25] and utilizes a set of well-defined molecular probes with widely varying sizes to determine pore dimensions [14]. The pore-size distribution of silica-based monolithic columns was characterized with inverse size-exclusion chromatography thoroughly [26–28].

In this work, I used inverse size-exclusion chromatography to describe accessible pore volume of particulate and monolithic stationary phases. For this, I have determined accessible porosity from size-exclusion calibration data and correlated it with the size of the pores from which individual compounds are excluded. Accessible porosity has been also compared to column efficiency for both small alkylbenzenes and large polystyrene standards. Presented data provide overall information about porous structure and efficiency of both particulate and monolithic stationary phases. Moreover, in case of polymer-based monoliths, they offer simple and straightforward control over the preparation process and can be used in future development of monolithic stationary phases with tailored porous properties and column efficiency.

2. Experimental

2.1. Chemicals and materials

Polyimide-coated 320 μm i.d. fused silica capillaries were purchased from Agilent (Palo Alto, CA, USA). 3-(Trimethoxysilyl) propyl methacrylate, sodium hydroxide, hydrochloric acid, 1,4-butanediol, 2,2′-azobisisobutyronitrile (AIBN) and alkylbenzenes (benzene, toluene, etylbenzene, propylbenzene, butylbenzene, amylbenzene) were purchased from Fluka (Buchs, Switzerland). Butyl methacrylate, lauryl methacrylate, ethylene dimethacrylate, tetraoxyethylene dimethacrylate, styrene, vinylbenzyl chloride (mixture of 3- and 4-isomers), divinylbenzene (technical grade), 1-propanol, 1-dodecanol, acetone, aluminum chloride, 1,8-diaminooctane, 1,6-dichlorohexane, and twelve polystyrene standards with molar masses ranging from 500 to 1 800 000 were obtained from Sigma–Aldrich (St. Louis, MI, USA). Tetrahydrofuran for gradient HPLC were from Merck (Darmstad, Germany). Distilled water was purified in a DEMIWA 5ROI station (Watek, Ledeč nad Sázavou, Czech Republic).

2.2. Columns

The commercially available columns (Zorbax 300SB-C18, 75 mm × 2.1 mm, particle size 5 μm, Agilent, Palo Alto, CA, USA; Poroshell 300SB-C18, 75 mm × 2.1 mm, particle size 5 μm with 0.25 μm layer of the porous stationary phase, Agilent, Palo Alto, CA, USA; and Chromolith Performance RP-18e, 100 mm × 4.6 mm, Merck, Darmstadt, Germany) have been used as received.

Monolithic capillary columns have been prepared according the protocols published previously [16,29]. The inner wall of the capillary surface has been first modified by 3-(trimethoxysilyl) propyl methacrylate prior the polymerization reaction [30]. Then, monoliths were prepared in capillaries using in situ radical reaction of particular polymerization mixture at various temperatures and times, as shows Table 1. The polymerization mixtures were sonicated for 10 min and filled in the vinylized capillaries. Both ends of the capillary were sealed with stoppers made from GC septa and the capillary was placed in a thermostated bath.

Columns 8–15 have been further modified by post-polymerization hypercrosslinking modification. While polymerization reaction proceeded to full conversion for columns 8–12, the polymerization reaction has been terminated after 2, 4, and 6 h for columns 13–15. Prepared generic monoliths have been first swelled in 1,6-dichlorohexane (columns 8–12) or tetrahydrofuran (columns 13–15) for 2 h. Then, hypercrosslinking modification has been performed at various reaction times using Friedel–Crafts alkylation in the presence of 5% $AlCl_3$ in 1,6-dichlorohexane for columns 8–12 [29] or by using nucleophilic substitution with 3% 1,8-diaminooctane in tetrahydrofuran at 95 °C for 2 h on columns 13–15 [31].

For further details of stationary phase surface modification see Table 1.

2.3. Instrumentation

A Thermo Scientific Dionex Ultimate 3000 RSLC system (Sunnyvale, CA, USA) equipped with an autosampler and a 45 nL UV detection cell was used for the characterization of the monolithic capillary columns with injection volume of 20 nL. The capillary columns were connected directly to injector and hyphenated to an UV detector by built-in capillary. In case of commercially available columns 1–3, fused-silica connecting capillaries (500 mm × 0.05 mm and 50 mm × 0.05 mm) have been used to connect separation column with injector and detector, respectively. For commercially available columns, a 180 nL UV detection cell and injection volume of 40 nL were used. Pure tetrahydrofuran was employed as a mobile phase for all experiments. Elution times and peak widths at the half height were determined by Chromeleon 7 Chromatography Data System.

Since proposed protocol is based on an accuracy of retention time determination, I have tested a stability of sample injection on column 15 which preparation combines both early termination of polymerization reaction and hypercrosslinking modification. The smallest polystyrene standard with molar mass of 500 has been repeatedly injected on 157 mm long capillary column ($n = 25$) and elution time, peak width at its half height, and column back pressure have been recorded. The instrumentation and column tested showed very good stability with values of retention time of 5.39 ± 0.01 min, peak width at half height of 0.92 ± 0.04 min, and back-pressure of 18.58 ± 0.52 MPa, respectively.