Journal of Chromatography A, 1325 (2014) 72–82

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Detailed characterization of the kinetic performance of first and second generation silica monolithic columns for reversed-phase chromatography separations

Deirdre Cabooter[a,*], Ken Broeckhoven[b], Roman Sterken[a], Alison Vanmessen[a], Isabelle Vandendael[c], Kazuki Nakanishi[d], Sander Deridder[b], Gert Desmet[b]

[a] KU Leuven, Department of Pharmaceutical Sciences, Pharmaceutical Analysis, Herestraat 49, 3000 Leuven, Belgium
[b] Vrije Universiteit Brussel, Department of Chemical Engineering, Pleinlaan 2, 1050 Brussels, Belgium
[c] Vrije Universiteit Brussel, Faculty of Engineering, Materials and Chemistry, Pleinlaan 2, 1050 Brussels, Belgium
[d] Kyoto University, Department of Chemistry, Sakyo-ku, Kyoto 606-8502, Japan

ARTICLE INFO

Article history:
Received 3 September 2013
Received in revised form
20 November 2013
Accepted 26 November 2013
Available online 1 December 2013

Keywords:
Second generation silica monoliths
Kinetic performance
Characteristic length
Flow resistance

ABSTRACT

The kinetic performance of commercially available first generation and prototype second generation silica monoliths has been investigated for 2.0 mm and 3.0–3.2 mm inner diameter columns. It is demonstrated that the altered sol–gel process employed for the production of second generation monoliths results in structures with a smaller characteristic size leading to an improved peak shape and higher efficiencies. The permeability of the columns however, decreases significantly due to the smaller throughpore and skeleton sizes. Scanning electron microscopy pictures suggest the first generation monoliths have cylindrical skeleton branches, whereas the second generation monoliths rather have skeleton branches that resemble a single chain of spherical globules. Using recently established correlations for the flow resistance of cylindrical and globule chain type monolithic structures, it is demonstrated that the higher flow resistance of the second generation monoliths can be entirely attributed to their smaller skeleton sizes, which is also evident from the external porosity that is largely the same for both monolith generations ($\varepsilon_e \sim 0.65$). The recorded van Deemter plots show a clear improvement in efficiency for the second generation monoliths (minimal plate heights of 13.6–14.1 μm for the first and 6.5–8.2 μm for the second generation, when assessing the plate count using the Foley–Dorsey method). The corresponding kinetic plots, however, indicate that the much reduced permeability of the second generation monoliths results in kinetic performances (time needed to achieve a given efficiency) which are only better than those of the first generation for plate counts up to $N \sim 45{,}000$. For more complex samples ($N \geq 50{,}000$), the first generation monoliths can intrinsically still provide faster analysis due to their high permeability. It is also demonstrated that – despite the improved efficiency of the second generation monoliths in the practical range of separations ($N = 10{,}000$–$50{,}000$) – these columns can still not compete with state-of-the-art core–shell particle columns when all columns are evaluated at their own maximum operating pressure (200 bar for the monolithic columns, 600 bar for core–shell columns). It is suggested that monolithic columns will only become competitive with these high efficiency particle columns when further improvements to their production process are made and their pressure resistance is raised.

1. Introduction

The first generation of monolithic silica columns has been on the market for more than a decade now [1–4]. In contrast to packed bed columns, monolithic columns consist of a single, continuous porous skeleton with large throughpores. The large throughpores result in a high external porosity (typically 70–80%, whereas this value is rather around 38% for packed beds) leading to large permeability values, which can be several times larger than that of columns packed with 5 μm particles [5]. This high permeability allows monolithic columns to be used at high linear velocities, making them extremely suitable as second dimension columns in e.g. online two-dimensional separation set-ups [6]. Due to their high permeability, monolithic columns can also be coupled to long column lengths resulting in unprecedented efficiencies [7].

The small size of the silica skeletons (1–2 μm) on the other hand, results in efficiencies comparable to those of 3–5 μm

* Corresponding author. Tel.: +32 016 32 34 42; fax: +32 016 32 34 48.
E-mail address: deirdre.cabooter@pharm.kuleuven.be (D. Cabooter).

0021-9673/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2013.11.047

particle columns, while the small mesopores give rise to a large sample capacity per unit adsorbent volume [8].

Despite these promising features, monolithic silica columns have not been able to compete with the novel generation of packed particle columns which was commercialized around the same time [9–11]. This lack in performance has mainly been attributed to the fabrication process, which fails to deliver radially homogeneous 4.6 mm inner diameter (I.D.) columns, an observation that was recently confirmed by an in-depth evaluation of the eddy diffusion term in first generation monolithic columns [12].

Macroporous silica monoliths are typically produced from alkoxysilanes using a sol–gel method in the presence of water-soluble organic polymers. The fabrication starts by hydrolysis and polycondensation of high-purity alkoxy silicon derivatives, such as tetramethoxysilane (TMOS) or tetraethoxysilane (TEOS), to form a sol. Adding water and a catalyst starts a reaction process resulting in gel formation. Simultaneously with the gel formation, spinodal decomposition occurs and phase separation takes place between the silica-rich and water-rich phase, representing the future silica skeletons and throughpores, respectively. To manipulate phase separation and thus control the pore size of the gel, a porogen such as polyethylene glycol (PEG) (or polyethylene oxide (PEO)) can be used. By varying the concentration of the porogen, the size of the throughpores can be controlled. Aging in a siloxane solution increases the stiffness and strength of the gel by adding new monomers to the silica skeleton. Adding ammonium to the aging solution, mesopores are formed. The amount and size of the mesopores depend on the concentration of ammonium. The gel is finally dried by capillary pressure, causing the gel to shrink. After drying, the monolithic rod is cladded with PEEK to ensure no void spaces remain around the monolith [13–16].

To improve the performance of the first generation monoliths, alterations to the production process have been made, resulting in the production of so-called second generation monoliths. In 2006, second generation capillary monoliths with an increased structural homogeneity and improved efficiency were obtained by varying the concentration of TMOS and PEG. This resulted in monolithic columns yielding plate heights of 4–5 μm [17]. Very recently, the step toward normal bore second generation monoliths has been made by making further modifications to the sol–gel process. Merck launched commercially available 4.6 mm I.D. second generation monoliths in 2011 that were produced using an increasing amount of porogen [18]. The performance of these second generation monoliths has been evaluated for the analysis of small molecules and large biomolecules by several authors [19–23].

Recently, Kyoto Monotech released prototype samples of 2.0 mm I.D. and 3.2 mm I.D. second generation monoliths. These monoliths are produced using poly-acrylic acid (HPAA) as a phase-separation inducer instead of PEG. PEG is distributed to the silica-rich phase, resulting in the formation of a hydrophobic layer at the surface of the gelling phase due to the specific adsorption of PEG chains onto surface silanol groups of silica oligomers [24]. This process is more pronounced when a hydrophobic mold is used in which case a dense layer, called the skin layer, is formed on the outermost part of the gelled silica rods. The formation of this layer results in a deformation of the framework of the gel before the actual gelation. Deformation of the gelling skeleton beneath the skin layer may also occur, resulting in structural inhomogeneities in the outermost part of the column. Both have a negative effect on the performance of the column. HPAA on the other hand, is distributed to the solvent phase and not the silica rich-phase upon phase separation. This results in less formation of skin layer and deformation of the skeleton in the vicinity of the mold wall, hence resulting in structures with an improved radial homogeneity. It is also easier to produce monolithic columns with a much smaller domain size using HPAA, which will result in improved column efficiency, however, at the cost of an increased column backpressure. The cladding process of the second generation monoliths is also different as it uses a partially molten glass tube, resulting in a less prominent skin layer and hence a smaller loss of efficiency than a hydrophobic tube [25,26].

The present study aims at evaluating the kinetic performance of first and second generation monoliths with similar dimensions by taking efficiency and permeability simultaneously into account, as opposed to other studies were the efficiency and permeability of both generation monoliths has been evaluated separately [19–23]. For this purpose, the kinetic plot method is used. Kinetic plots are obtained by transforming experimentally obtained van Deemter (u_0, H) and permeability (K_{v0}) data using the following equations [27,28]:

$$N = \left(\frac{\Delta P}{\eta}\right)\left[\frac{K_{v0}}{u_0 H}\right] \quad (1)$$

$$t_0 = \left(\frac{\Delta P}{\eta}\right)\left[\frac{K_{v0}}{u_0^2}\right] \quad (2)$$

wherein u_0 (m/s) is the linear velocity of the mobile phase, H is the plate height (m), K_{v0} (m^2) the permeability of the column, ΔP (Pa) the pressure drop and η (Pa s) the viscosity of the mobile phase.

A typical kinetic plot of plate count N versus column dead time t_0 (min) or retention time t_R (min) ($t_R = (1 + k)\cdot t_0$, with k the retention factor of the analyte) shows the efficiency N obtained in a certain time t in a column that is exactly long enough to generate a specific pressure ΔP at a given velocity u_0. The pressure drop ΔP used in Eqs. (1) and (2) is the maximum pressure that can be delivered by the instrument or the maximum pressure the column can withstand and gives an idea of the ultimate performance limit of the support. In the present study, the maximum pressure was set at 200 bar for the monolithic columns.

A detailed investigation of the pressure drop characteristics of both generation monoliths is reported as well. For this purpose, total pore blocking (TPB) experiments have been performed to accurately determine the external porosity ε_e of the columns [29,30] and computationally determined correlations have been used to relate the experimentally determined permeability and porosity values [31]. The accuracy of these correlations is moreover demonstrated experimentally for the first time for monoliths with single globule chain-type skeleton branches and cylindrical skeleton branches.

Finally, the performance of first and second generation monoliths is compared to that of state-of-the-art porous particles and evaluated at different operating pressures.

2. Experimental

2.1. Chemicals and columns

Propiophenone, butyrophenone and benzophenone were obtained from Sigma–Aldrich (Steinheim, Germany), thiourea from Acros (Geel, Belgium) and potassium iodide (KI) from VWR (Leuven, Belgium). Milli-Q water was prepared using a Milli-Q gradient water purification system from Millipore (Bedford, MA, USA). HPLC grade Acetonitrile (ACN) was purchased from Fisher Chemicals (Erembodegem, Belgium), ammonium acetate from Sigma–Aldrich and glacial acetic acid from Merck (Darmstadt, Germany).

The first generation monolithic columns (Chromolith Performance RP-18, 2.0 mm × 100 mm and 3.0 mm × 100 mm) were purchased from Merck. The second generation monolithic columns (3.2 mm × 50 mm, 3.2 mm × 100 mm, 2.0 mm × 50 mm and 2.0 mm × 100 mm) were kindly supplied by Prof. Nakanishi from Kyoto University. All monoliths had a maximum operating