



The performance of second generation silica monoliths, operating in parallel segmented flow mode



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ARTICLE INFO

Article history:

Received 19 August 2015

Accepted 24 August 2015

Available online 3 September 2015

Keywords:

Active flow technology

Second generation silica monolith

Radial heterogeneity

Parallel segmented flow

Wall effect

ABSTRACT

This study investigates the chromatographic performance of second generation monoliths in its conventional design, as well as replacing the standard outlet with an Active Flow Technology (AFT) assembly to minimise inefficient flow contributions. The AFT coupled monolith resulted in up to 50% improvement on the conventional monolith's efficiency and a 16% improvement in sensitivity. Exploitation of the current second generation monolith's silica infrastructure and AFT coupling in the parallel segmented flow (PSF) mode of operation would benefit high through-put analyses that demand highly efficient, sensitive, low volume detection, front end separation solutions utilizing conventional HPLC systems.

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1. Introduction

The second generation monolith was recently developed and studies on this column indicate a substantial improvement in the efficiency [1–8]. The physical and chemical properties that differentiate the second generation monoliths from the first generation include: macropore size was reduced to 1.1–1.2 μm (first generation 1.8–2 μm), the mesopore size increased to 14–16 nm (first generation 11–12 nm), total pore volume decreased to 2.9 mL/g (first generation 3.5 mL/g), and the surface area was reduced to 250 m^2/g (from 320 m^2/g) [1]. These structural changes were studied by Gritti and Guiochon, and showed that the 4.6 mm internal diameter (i.d.) second generation monolith [3] had plate heights at around 6.5 μm , yielding around 155,000 N/m, effectively three times that afforded by the 1st generation. They also noted a structural change in the bed morphology – a size reduction of 35% of the domain size in comparison to the 1st generation monolith, which effectively enabled the increase in efficiency. A significant reduction in the heterogeneity was apparent, common knowledge in monolithic technology [1–8], virtue of a 50% reduction in the overall eddy diffusion term. Although far more uniform than the 1st generation monolith, the bed still displayed signs of heterogeneity as un-retained species exhibited a higher plate height compared to more strongly retained species, especially at high velocity. The permeability of the

second generation monolith was designed to match conventional columns of the same dimension packed with fully porous $>6 \mu\text{m}$ particles.

The general conclusion made by Gritti and Guiochon was that the second generation monolith performed at around the same level of performance as sub 3 μm core shell particles and sub 2 μm fully porous particles [3]. The practical benefits of the second generation monolith include the ability to stay within the pressure limits of the conventional HPLC system.

The present study follows from prior works that evaluated the performance of the first generation silica monolith modified with active flow technology fittings [9,10]. These earlier studies demonstrated that the efficiency of the first generation silica monolith could be improved by around 100% when operated in either parallel segmented flow mode [9], or curtain flow mode [10]. These gains in performance obtained when AFT fittings are utilised on the monolith are achieved because the concept of active flow technology is to extract only solutes that eluted from the most efficient region of the column bed [9–22], in fact, provide flow splitting in the radial direction.

The use of AFT with silica monoliths has so far included only the first generation analytical scale silica monolith (4.6 mm i.d.) operated in either parallel segmented flow (PSF) mode [9] where the AFT ensemble is fitted on the column outlet; and curtain flow (CF) mode [10] where the AFT fittings are coupled at both the inlet and outlet of the column. In PSF mode an increase in efficiency of 111% compared to that of the conventional monolith was observed, while maintaining the sensitivity [9]. The efficiency of the CF monolith was observed to increase by around 130%, with a gain in sensitivity of 250%, compared to the

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conventional format monolith [10]. These studies revealed the substantial effect that radial bed heterogeneity within the monolith had on the performance of the monolith, and importantly, once this heterogeneity is removed, efficiency increases significantly [9,10]. Despite the use of active flow technology on the first generation silica monoliths, the efficiency gains did not reach the range achieved by the conventional second generation monolith. Hence it is the aim of this study to further explore AFT in concert with the second generation silica monolith.

2. Experimental

2.1. Chemicals

HPLC grade acetonitrile was purchased from Fisher Chemicals (Loughborough, UK). Milli-Q water (18.2 M Ω cm) was prepared in-house and filtered through a 0.2 μ m filter. Theophylline, toluene, propylbenzene, and butylbenzene were all purchased from Sigma Aldrich (Dorset, UK). All materials were used as received. All mobile phases were prepared volumetrically and used without further filtration.

2.2. Equipment

Chromatographic investigations were performed on an Ultimate 3000 RSLC dual pump instrument with UV detection, running Chromeleon 7.0 software.

The second generation analytical scale silica monolith – Chromolith High-Resolution 100 \times 4.6 mm i.d., was supplied by Merck (Darmstadt, Germany). The adapted end-fittings and active flow frits were machined in-house by Thermo Fisher Scientific (Runcorn, UK). The AFT frit design is consistent with previous studies [9,10].

A superficially porous particle packed column, Accucore C18 2.6 μ m d_p , 100 \times 4.6 mm i.d., was supplied by Thermo Fisher Scientific (Runcorn, UK).

A GJC HPLC Liquid Flowmeter (Cheshire, UK) was used to measure the liquid flow and outlet segmentation ratios.

2.3. Column efficiency and asymmetry measurements

All performance metrics were measured using the Chromeleon 7.0 software. Theoretical plates were calculated using the USP method, which is based on the width of the peak at half height and also using the second moment (SM) method, which is more sensitive to peak tailing and asymmetrical behaviour.

The second moment method was calculated where $N = (\mu'_1/\mu'_2)^2$, where N is the number of theoretical plates, μ'_1 is the first central moment, or retention time, and μ'_2 is the second central moment, or variance of the peak. The second moment method integrates the peak width at 5σ across the base of the peak and is more susceptible to indicate tailing, co-elution or asymmetrical phenomena [23].

The asymmetry (tailing factor) = $a/2b$, where a is the peak width measured at 5% height and b is the width of the first portion of the peak measured at 5% of the peak height [24].

2.4. Standard and sample preparation & chromatographic conditions

The standard test mixture was prepared using the mobile phase and contained theophylline (0.02 mg/mL), toluene (0.30 mg/mL), propylbenzene (0.45 mg/mL), and butylbenzene (0.60 mg/mL). Chromatographic behaviour of these solutes was assessed under isocratic conditions. The isocratic mobile phase of 60:40 acetonitrile:water (v/v), was pre-prepared and delivered through a single pump for the standard and PSF monoliths. The flow rate was kept constant at 2.0 mL/min. The PSF outlet segmentation ratios were tested at 13–68% (through radial central outlet port) and were controlled by careful addition of linear pressure restrictors, in the form of viper fittings (Thermo Scientific,

Germerring, Germany). Injections were performed in triplicate and at ambient room temperature. Injection volumes were set at 5 μ L.

Decaffeinato Intenso coffee samples were prepared as a 30 mL shot using a Nespresso cartridge coffee machine. These samples were diluted fourfold and filtered through a 0.2 μ m nylon filter. The coffee was analysed using gradient elution conditions on both the AFT monolithic column and the Accucore column. In the case of the AFT monolithic column the outlet segmentation ratio was set at 25% from the radial central exit port and 75% from the peripheral port. Separation conditions on both columns were identical, specifically, the initial mobile phase composition was 100% water, followed by a linear gradient running to 100% methanol at a rate of 2.5% per minute. The gradient was initiated 1 min after injection. At 100% methanol, the composition was held for 3 min then returned to the initial conditions in 1 min. Prior to any injection, either column was equilibrated with five column volumes of the initial mobile phase composition. The mobile phase flow rate was 2 mL/min. The sample injection volume was 5 μ L and detection was set 280 nm.

3. Results and discussion

3.1. USP efficiency

The USP efficiency is compared for the AFT monolith in PSF mode and the conventional column in Fig. 1. The percentage volumetric flow directed to the detector, also known as the segmentation ratio, is simply the percentage of the bulk flow taken from the radial central port of the AFT outlet. The USP approach integrates the peak at half height. This method is less sensitive to peak broadening that occurs closer to the baseline [23]. The percent, relative standard deviation in the USP efficiency measurements (%RSD) for all solutes was $\leq 0.6\%$ and $\leq 0.7\%$ for the PSF and conventional column, respectively.

The conventional second generation monolith had an efficiency of 161,000 N/m for butylbenzene (retention factor (k) = 5), 170,000 N/m for propylbenzene (k = 3) and 175,000 N/m for toluene (k = 1). When this same monolith was utilised in PSF mode, the efficiency improved, irrespective of outlet segmentation ratio. The largest increases in efficiency were 28% (206,000 N/m) for butylbenzene, 24% (210,000 N/m) for propylbenzene, and 14% (~200,000 N/m) for toluene when the outlet segmentation ratio was 32%. When the outlet segmentation ratio was increased to 43% the gain in efficiency for toluene was almost unaffected, but reached its highest level of performance, recording a 15% gain. Effectively, the outlet segmentation ratio that provided the highest level of

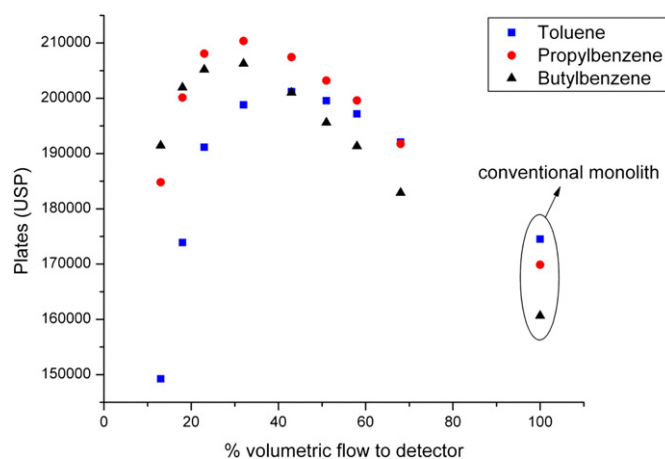


Fig. 1. USP efficiency (N/m) as a function of % flow to the PSF central outlet port, sent to the detector. The AFT monolith was only operated in PSF mode, for different outlet segmentation ratios that are compared to the conventional column. The comparison is made for toluene (blue), propylbenzene (red) and butylbenzene (green), 5 μ L of the standard mixture injected under isocratic conditions, 60:40 acetonitrile: water (v/v), at ambient temperature, at a flowrate of 2 mL/min.

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