Journal of Chromatography A, 1351 (2014) 56–60

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Assessing the performance of curtain flow first generation silica monoliths

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ARTICLE INFO

Article history:
Received 18 December 2013
Received in revised form 28 April 2014
Accepted 8 May 2014
Available online 17 May 2014

Keywords:
Active flow technology
Silica monolith
Parallel segmented flow
Curtain flow

ABSTRACT

Analytical scale active flow technology first generation silica monolithic columns kitted out in curtain flow mode of operation were studied for the first time. A series of tests were undertaken assessing the column efficiency, peak asymmetry and detection sensitivity. Two curtain flow columns were tested, one with a fixed outlet ratio of 10% through the central exit port, the other with 30%. Tests were carried out using a wide range in inlet flow segmentation ratios. The performance of the curtain flow columns were compared to a conventional monolithic column. The gain in theoretical plates achieved in the curtain flow mode of operation was as much as 130%, with almost Gaussian bands being obtained. Detection sensitivity increased by as much as 250% under optimal detection conditions. The permeability advantage of the monolithic structure together with the active flow technology makes it a priceless tool for high throughput, sensitive, low detection volume analyses.

1. Introduction

active flow technology (AFT) [1] was largely designed to overcome the problems caused by wall effects and radial packing heterogeneity that limit the performance of current HPLC column technology [2–5]. In addition to improving the efficiency of chromatography columns other advantages in this column technology also transpired, for example, when AFT columns were coupled to flow limiting detectors, i.e., the mass spectrometer, through-put could be dramatically increased since the volume load to the detector could be reduced [6]. In addition, AFT columns kitted out in the curtain flow mode of operation provided for a substantially higher level of sensitivity in detection, since the analyte was constricted in the central region of the column [6–11]. Parallel segmented flow columns enabled multiplexed detection processes to be employed, which expanded the amount of information that could be collected on the sample within a single analysis [12–17].

To date, the performance of AFT columns has been tested on particle packed column formats only, but in scales that range from the preparative scale (21 mm internal diameter (i.d.)) down to narrow bore columns (2.1 mm i.d.) [18]. The gain in separation performance across this range of operation was reviewed recently [1] and thus, details relating to specific aspects of the performance of AFT columns need not be repeated here. It is the aim of the current study, to demonstrate the benefits AFT when applied to first generation silica based monolithic columns. These columns are perhaps the ideal candidate for the active flow technology, since the bed structure is such that peak tailing is substantial, and this has limited the performance of the monolithic column. Much of this tailing has been ascribed to the column wall effects and radial heterogeneity of the stationary phase bed [19,20].

Even though peak tailing has limited to some extent the performance of the first generation monolith, which was the driver towards the development of the second generation silica monolith, the first generation monolith has proven to be a useful tool for chromatographer's who require high throughput separations since these beds offer a high degree of permeability and yield efficiency approaching 5-μm particle packed columns when the number of theoretical plates (N) is measured at half height, although substantially less efficiency is apparent when tailing is considered. Monolithic columns have been the focus of two independent and extensive reviews by Cabrera [21] and Guiochon [22]. Both highlighted their main advantage; high permeability and fast mass transfer kinetics (reduced c-term) [21,22]. The benefits of throughput are substantial for the first generation silica monolith, and it is this additional factor that promotes the potential of utilising

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http://dx.doi.org/10.1016/j.chroma.2014.05.025
0021-9673/

active flow technology in combination with the silica monolith. Recently we studied the performance of parallel segmented flow first generation silica monoliths, and we showed that the separation performance, based on *N*, could be increased by as much as 110% [23]. In the present study, we extend this work by testing the performance of curtain flow first generation silica monoliths. Here we compare the performance of commercially available first generation silica monoliths to the specially prepared first generation curtain flow monoliths, and also to our prior study conducted on parallel segmented flow (PSF) first generation silica monoliths. The chromatographic performance was based on the metrics; *N*, asymmetry and sensitivity. Detailed descriptions of the curtain flow concept can be found in refs. [1,6–11] and need not be discussed here further.

2. Experimental

2.1. Chemicals

HPLC grade acetonitrile was purchased from Fisher Chemicals (Loughborough, UK). Milli-Q water (18.2 MΩ cm^{-1}) was prepared in-house and filtered through a 0.2 μm filter. Theophyline, 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 system dual pump instrument, using Chromeleon 7.0 software.

The first generation analytical scale silica monolith – Chromolith Performance 100 × 4.6 mm i.d. was supplied by Merck Millipore (Darmstadt, Germany). The adapted end-fittings and active flow frits were machined in-house by Thermo Fisher Scientific (Runcorn, UK). The AFT frit design was consistent with the previous study [23].

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

2.3. Column efficiency 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. The SM method integrates the peak at 5σ across the peak base and hence is more susceptible to peak tailing and asymmetrical phenomena.

2.4. Standard mixture sample preparation and chromatographic conditions

The standard mixture was prepared using the mobile phase and contained theophyline (0.02 mg/mL), toluene (0.30 mg/mL), propylbenzene (0.45 mg/mL), 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 columns. The flow rate was kept constant at 2.0 mL/min. In experiments involving the CF first generation silica monolith the outlet segmentation ratios were set at either 10 or 30% mobile phase flow from the central outlet exit port. These values were chosen based on the findings from the previous study [23] and were controlled by careful adjustment of the pressure differential between the peripheral and central exit ports. The CF inlet segmentation ratios were tested at 10–99% directed through the central port. The flow differentials between the inlet peripheral ports and the inlet central port was precisely controlled using the dual pump configuration of the Ultimate 3000 system. Injections were performed in triplicate and at ambient room temperature. Injection volumes were set at 5 μL.

3. Results and discussion

3.1. Column efficiency

The plot in Fig. 1 details the efficiency based on the elution of butylbenzene from the first generation silica monolith operated in the various modes, where curve (a) describes the elution

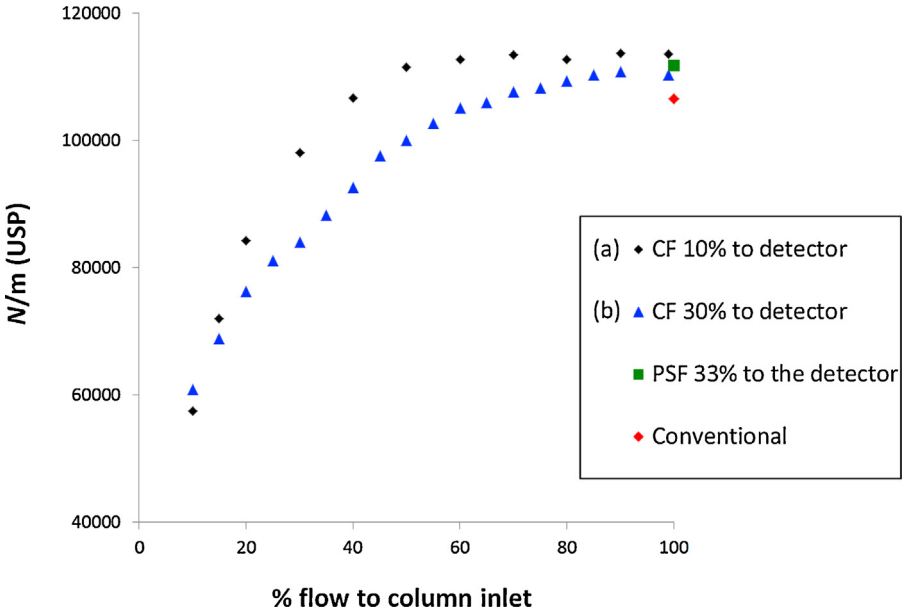

Fig. 1. USP efficiency (N/m) as a function of % flow to the column inlet. The AFT monoliths with various fixed outlet segmentation ratios: (a) CF 10% outlet ratio, (b) CF 30% outlet ratio and the PSF 33% outlet ratio, compared to the conventional column. The comparison is for butylbenzene, 5 μL of the standard mixture injected under isocratic conditions, 60:40 acetonitrile:water (v/v), at ambient temperature, at a flowrate of 2 mL/min.