



Enhancing the separation performance of the first-generation silica monolith using active flow technology: Parallel segmented flow mode of operation



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ABSTRACT

Active flow technology (AFT) columns are designed to minimise inefficient flow processes associated with the column wall and radial heterogeneity of the stationary phase bed. This study is the first to investigate AFT on an analytical scale 4.6 mm internal diameter first-generation silica monolith. The performance was compared to a conventional first-generation silica monolith and it was observed that the AFT monolith had an increase in efficiency values that ranged from 15 to 111%; the trend demonstrating efficiency gains increasing as the volumetric flow to the detector was decreased, but with no loss in sensitivity.

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1. Introduction

Active flow technology (AFT) is the term that encompasses a range of new chromatography columns that are designed to eliminate wall effects, minimise solute band broadening due to radial heterogeneity [1–6] and decrease the peak volume of eluting components. Studies detailing the practical gains in separation performance of AFT columns have so far been demonstrated on particle-packed column formats from the preparative scale down to analytical scale [7–17], and more recently into narrow bore columns [18]. The results have shown conclusively that substantial gains in efficiency are obtained, although the gains cannot be generalised as they are dependent on numerous factors, which include column length, flow rate, particle diameter, the degree of retention and the segmentation ratio. Nevertheless, the magnitude of the gain in chromatographic performance is typified by the results reported on analytical scale columns packed with reversed phase silica particles, which were in the order of 50% in efficiency (N) in comparison to conventional columns of the same internal diameter (i.d.) [10]. Further details on the performance gains in AFT columns

have been published prior and need not be covered again here. It is the aim of the current study, however, to demonstrate that AFT columns benefit the performance of first-generation silica-based monolithic columns.

The first-generation monolith has proven to be of immense value in the chromatographer's toolbox, and has been the focus of extensive reviews [19,20]. Their main advantage is illustrated through their typical van Deemter curve having a lower c term, maintaining high chromatographic efficiency at high linear velocities due to the increased permeability of these beds afforded by their porous structure [19,20]. Thus, the throughput benefits are substantial and the performance at high velocities is a useful attribute for maximising peak capacity [21]. However, one limitation of the first-generation monolith is that they suffer from radial heterogeneity [22–25], which appears to generate substantial band tailing and therefore decreases the column's efficiency and peak capacity.

A recent study by Gritti and Guichon showed a tight relationship between the pore geometry (average domain size = sum of the average mesopore and macropore) with the achievable efficiency value of analytical scale silica monoliths [24]. One of the main findings from Gritti and Guichon's study was an increase in almost three times the efficiency value (55,000–155,000 N/m) was achieved following a 35% reduction in the structural average

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domain size (3.3–2.1 μm), obtained between the first and second-generation silica monoliths [24]. A consequence of reducing the domain size is, however, a decrease in bed permeability, which decreases the maximum operating pressure, hence reducing the maximum operating flow rate of the second-generation monolith [24]. The aim of this study is to couple the first-generation silica monolith with AFT fittings to overcome radial heterogeneity and thus truly realise the separation potential of these columns, maintaining the permeability advantage that they offer against particle-packed columns.

Within the suite of columns referred to as AFT columns, there are two modes of operation termed: parallel segmented flow (PSF) and curtain flow (CF) [16]. In the PSF mode of operation, the AFT fitting and frit ensemble is fixed to only the outlet of a conventional column, while in the CF mode of operation, the AFT fitting and frit ensemble is fixed to both the column inlet and the column outlet. Effectively, the AFT end-fitting ensemble provides the user with greater control in maximising the column efficiency by being able to tune the proportion of the column that is actually used for analytical purposes. Effectively, a “virtual” and wall-less column is established, which overcomes the limitations associated with column-bed radial heterogeneity [12]. The less mobile phase that is allowed to elute from the central exit port of the column, the smaller the internal diameter of the “virtual” column [12]. Operation in the CF mode has an additional advantage because sample can be loaded specifically into the central core region of the column inlet, which provides greater sensitivity in addition to creating a wall-less column. Irrespective, the AFT column in both modes minimises the inefficient peak-broadening processes caused by radially heterogeneous beds. For more detailed explanation of how AFT columns are operated, in a practical sense, readers are referred to Refs. [7–12,14–17].

In the current study, the chromatographic performance of the conventional first-generation silica monolith will be compared to an AFT monolith. To do this objectively, the conventional monolith once tested was converted to an AFT monolith operating in a PSF mode. This was achieved by designing AFT fittings specifically for the silica monolith. Chromatographic performance; N , asymmetry and sensitivity are used to gauge the practical gains of the AFT first-generation monolith compared to the conventional first-generation monolith.

2. Experimental

2.1. Chemicals

HPLC grade acetonitrile was purchased from Fisher Chemicals (Loughborough, UK). Milli-Q water ($18.2\text{ M}\Omega\text{ cm}^{-1}$) was prepared in-house and filtered through a $0.2\text{-}\mu\text{m}$ 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 system dual pump instrument, controlled using Chromeleon 7.0 software.

The first-generation analytical scale silica monolith, chromatolith performance $100 \times 4.6\text{ mm i.d.}$, was supplied by Merck (Darmstadt, Germany). The AFT end-fitting ensembles were machined in-house by Thermo Fisher Scientific (Runcorn, UK). The AFT frit design had an average pore size of $2\text{ }\mu\text{m}$. Segmentation ratios were varied by changing the relative back pressures between the central exit port

and the peripheral exit ports simply by altering the length of the respective tubings. This is detailed in Refs. [7–12,14–16].

2.3. Column Efficiency Measurements

Column efficiency was measured using two approaches within the Chromeleon 7.0 software. The first method was the commonly employed United States Pharmacopeia (USP) approach, which is based on the measurement of peak width at half height, while the second method employed the measurement of the peak variance, a method column referred to as the statistical moment (SM) method, or simply the variance method. This second method integrates the peak at a width of 5σ across the peak base and hence is more sensitive to peak tailing, co-elution or asymmetrical phenomena.

2.4. Standard Mixture Sample Preparation and Chromatographic Conditions

The standard 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 prepared and delivered through a single pump for the standard and PSF tests. The flow rate was kept constant at 2.0 mL/min. PSF segmentation ratios tested were in the range 14–78%. The outlet flow rate ratios were controlled by careful addition of linear pressure restrictors, in the form of viper fittings (Thermo Fisher Scientific, Germering, Germany). Injections were performed in triplicate and at ambient room temperature. Injection volumes were set at $5\text{ }\mu\text{L}$.

3. Results and Discussion

3.1. USP Efficiency

Fig. 1 is a visual comparison of the efficiency measurements calculated using the USP methodology, on the conventional and PSF monoliths as a function of the outlet segmentation ratio, expressed as the percent flow from the column centre to the bulk detector.

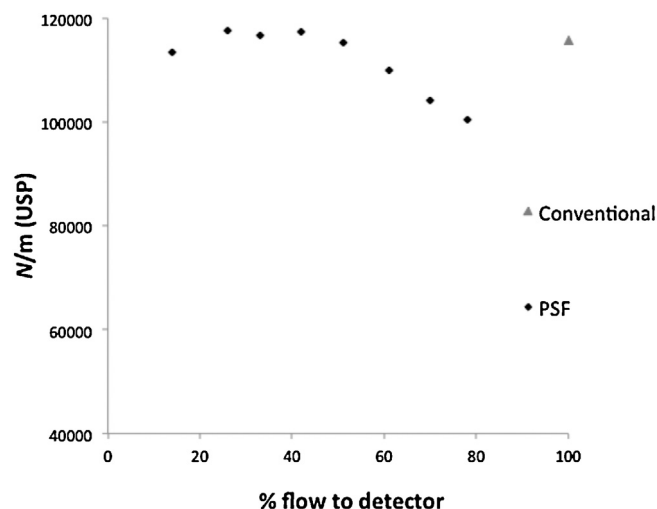


Fig. 1. USP efficiency (N/m) as a function of % flow to the detector (outlet segmentation). The conventional column (grey) and the PSF monolith (black) comparison for propylbenzene. Five microlitres of the standard mixture injected under isocratic conditions, 60:40 acetonitrile: water (v/v), at ambient temperature, at a flow rate of 2 mL/min for the conventional and PSF, respectively.

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