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Gradient elution chromatography with segmented parallel flow column technology: A study on 4.6 mm analytical scale columns☆

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ABSTRACT

A new column format known as parallel segmented flow has recently been introduced, whereby improvements in column performance are observed. These improvements are achieved via the separation of eluent from the column core from that of the column wall region. The segmentation of flow is accomplished immediately as the eluent exits the column through the use of a multi-channel end fitting. The ratio of flow exiting through the column central port relative to the peripheral ports, known as the segmentation ratio, can be tuned to optimise chromatographic performance. Investigations into the use of parallel segmented flow chromatography columns have demonstrated increased sensitivity and theoretical plates in analytical scale isocratic separations, but so far no studies have detailed the performance of these columns in gradient elution. The current study addresses the performance of parallel segmented flow columns in gradient elution, detailing the reproducibility of the gradient at various segmentation ratios and compares the performance to conventional columns. The study found that there was no observable difference in the gradient shape, or reproducibility of the gradient profiles generated at any segmentation ratio, tested on three different types of stationary phases. A separation of an 11-component test mixture verified that the primary advantage of parallel segmented flow columns was that the peak volume was reduced in proportion to the segmentation ratio.

1. Introduction

The quest for more efficient and more sensitive chromatographic separations has lead to the development of the parallel segmented flow column, the focus of the present study. It is now well known that solute transport through chromatography columns is not uniform: Variations in packing density [1–19], both radially and axially, and the generation of thermal gradients due to frictional heating [20–29] lead to the distortion of the band profile. While the variation in bed density in the axial direction is important since it increases the reduced plate height, its effect on modern HPLC columns is small, as high performance columns are now packed in short column formats and this axial heterogeneity extends in scales larger than the current dimensions [4]. Hence individual beds are now more likely to be essentially uniform in the axial direction. However, radial heterogeneity is far more detrimental to column performance since variations in the packing density across the column radius lead to variations in flow velocity. The bed density usually increases from the column centre to the wall region and as consequence flow is faster in the central region than near the wall. The solute band thus migrates along the column in a parabolic profile. Further degradation in separation performance occurs as a result of the 'wall effect' whereby a higher than average void fraction occurs at the wall because the particles and wall surface cannot bend to accommodate the other. Hence solute migrating in this section of the column region does so at higher velocity than the bulk.

The principle of the parallel segmented flow column involves the separation of mobile phase that elutes from the radial central region of the packed bed from that of the mobile phase that elutes near the wall section. The purpose of separating these two flow regions is to overcome the radial heterogeneity in the flow velocity of the migrating solute band, These contributing factors to the radial heterogeneity of the migrating solute band ultimately result in the band profile resembling a hollow bowl shape, rather than an ideal narrow disc. As a consequence more theoretical plates are required to separate in their entirety, bowls, rather than disks. However, if the requirement is only to separate the surfaces of the bowls at any specific point within the column (or at the column outlet) then the number of theoretical plates required is simply equivalent to

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that required to separate flat, uniform disks. This is in essence the reason why localised end column detection yields higher apparent separation efficiency [8–15,30–32].

To overcome the radial heterogeneity of solute migration a specially designed outlet fitting and frit was designed that separates, or segments, flow between the central and wall flow regions at the point of exit from the column. Such a fitting mimics the concept of localised end-column detection, but offers the advantages of bulk detection. A series of papers have been published that detail the design and operation of this type of column at the analytical [33,34] and preparative [35] scales and these studies also detail more specifically the heterogeneity of the packed bed. A feature of these new columns designs is that the proportion of flow that exits the column via either the inner region of the column, or peripheral region can be varied in order to fine tune the number of theoretical plates [33,35], the sensitivity in detection [33,35], or the volume of the elution band [33,34].

To date, studies detailing the performance of segmented parallel flow columns have been limited mainly to isocratic elution, however, gradient elution separations are routinely employed in the analysis of more complex samples, where there is a large variation in the range of retentivity on the stationary phase. Hence application of parallel segmented flow chromatography columns in gradient elution conditions is important.

We present here a study on the operation performance of analytical scale parallel segmented flow chromatography columns under the conditions of gradient elution. The applicability of parallel segmented flow columns to gradient elution was tested on three different columns; a C18, a cyano and a pentaflurophenyl stationary phase. Gradient rates were tested from 1% per minute to 10% per minute.

2. Experimental

2.1. Chromatography columns

Chromatography columns, reversed phase Hypersil GOLD, cyano and pentaflurophenyl chromatography columns (100 mm × 4.6 mm, 5 μm P_d) were supplied by ThermoFisher Scientific (Runcorn, Cheshire, United Kingdom). Specialised parallel segmented flow outlet fittings were made for this work by ThermoFisher Scientific. Details of these fittings are illustrated in Fig. 1, which shows a diagram of the end fitting and frit design. The end fitting contains four outlet ports, three that channel flow from the wall region, and a central port that captures flow from the radial centre of the column. A special annular frit aids in the efficiency of this segmentation process. The relative amount of mobile phase that elutes from either the inner or peripheral zone can be adjusted by changing the pressure drop between the peripheral ports and the central port. Details of this end fitting and its operation have been discussed in previous works where we studied the performance gains (*N* and sensitivity) in columns fitted with parallel segmented flow outlet fittings [33] and in columns fitted with a curtain flow inlet fitting and a parallel segmented flow outlet fitting [35], which effectively produced an infinite diameter column.

2.2. Chemicals and reagents

All mobile phases were prepared from HPLC-grade solvents purchased from Merck (Kilsyth, Victoria, Australia). All chemicals were commercially available. Caffeine, phenol, p-cresol, anisole, phenetole, naphthalene, sec-butylbenzene, butylbenzene, pentabenzene, hexylbenzene and 1-phenyloctane were purchased from Sigma–Aldrich (Castle Hill, New South Wales, Australia). Milli-Q water (18.2 MΩ cm^{-1}) was prepared in-house and filtered through a 0.2 μm filter.

2.3. Reagents

The standard 11-component test mixture was prepared in 100% HPLC-grade methanol at the concentrations ranging from 0.5 mol/L to 1.3 mol/L. The mixture was sonicated for 30 min and filtered through a 0.45 μm syringe filter (Merck Millipore, Kilsyth, Victoria Australia).

2.4. Chromatographic separation

Chromatographic experiments were performed using a Shimadzu LC-20ADvp chromatographic system (Shimadzu, Rydalmere NSW, Australia) equipped with a Shimadzu LC-20ADvp quaternary pump, Shimadzu SIL-10ADvp auto injector, Shimadzu SPD-M10Avp photo diode array detector and an on-line degassing module.

Gradient profiles and reproducibility data was acquired at 275 nm using either of two mobile phase conditions:

(1) Solvent A – 100% methanol, and solvent B – methanol + (0.0025% acetone (v/v)),
(2) Solvent A – 100% water and solvent B – methanol + (0.0025% acetone (v/v)).

Gradient reproducibility was then tested using initial conditions of 70% solvent A, 30% solvent B running to 20% solvent A, 80% solvent B, at the gradient rates as specified within the text. Analysis was performed in at least triplicate. Flow rates were always 1 mL/min. At least four segmentation ratios were tested on each of the three chromatography columns, as noted within the text as appropriate.

The gradient separation of the 11-component standard test mixture was performed using a 10% per minute gradient rate starting from 50% methanol, 50% water and finishing at 100% methanol. Injection volume was 10 μL and UV detection was performed at 254 nm. The separation was performed in duplicate using four different segmentation ratios as specified within the text.

3. Results and discussion

3.1. Segmented parallel flow in gradient elution

There are two primary requirements in order for gradient elution to be compatible with segmented parallel flow column technology:

1. Gradient curves must be reproducible, so that they can be employed in all aspects of separations and not restricted to isocratic elution conditions.
2. The segmentation ratio must not change throughout a gradient, as this could limit quantitative analyses.

Gradient reproducibility was investigated using two different tests. The first test was performed using mobile phase condition (1) as noted in Section 2.4. Under these conditions the only change in mobile phase composition was the percentage of acetone, which increased as the percent composition of solvent B increased. Acetone was the UV-tracer, the purpose of which was solely to visualise the nature of the gradient profile produced by the HPLC system. Gradient rates of 1, 2, 5, and 10% per minute and a step gradient were tested on each column set (either a conventional column, or the respective parallel segmented flow column). Segmentation ratios that were tested were: 25%, 40%, 50% and 70% of the total