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Curtain flow chromatography (‘*the infinite diameter column*’) with automated injection and high sample through-put: *The results of an inter-laboratory study*

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ABSTRACT

An inter-laboratory study was undertaken to test the analytical performance of curtain flow chromatography columns. Two laboratories undertaking the analysis independently tested the curtain flow columns under the same chromatographic conditions, using the same test solutes. In total three different types of HPLC systems were employed during these tests. Irrespective of the laboratory or the type of HPLC system employed, the outcome in the analytical performance for these curtain flow columns followed the same trends. The differences in the data sets were related to the type of the HPLC system employed, specifically the dead-volume of the system and amount of noise generated in baseline data.

The outcome from the study was that calibration curves derived from analyses undertaken on curtain flow columns were linear. The limits of quantification and detection were almost three times lower when curtain flow columns were used than when standard columns where used. The best results were obtained when the HPLC system was designed such that it could deliver a synchronised solvent flow through both the peripheral and central column entry ports.

1. Introduction

Aside from developments in particle technology, and the development of monolithic structures, during the period of time that HPLC columns have been manufactured there has been little change in their design. Largely the process of solute transport through packed beds has stayed the same. The column has always been a tube fitted with a frit at the inlet, perhaps also a distributor depending on the column scale, and an outlet fitting also containing a frit, the purpose of which is to retain stationary phase inside the column container. Flow enters the header through a single central port, and exits the column through a single central port at the outlet.

There is now a greater understanding of how the bed structure and the column geometry contribute to band broadening. A key factor that limits separation potential is the heterogeneity of the packing material that makes up the bed, and the consequential flow heterogeneity that results from differential bed densities, which varies across the radial cross section of the column [1–10]. This has been well described by numerous researchers and further discussion here is not warranted. In addition to the heterogeneity of the bed packing structure an effect long recognised as a factor that limits separation performance is the wall effect [7–10]. This has now been well described by numerous researchers and need not be covered in detail here. Nevertheless, what is important, and should be noted here, is that bands that migrate through packed beds in column tubes do so in distributions that resemble bowls, rather than thin flat discs [7–9]. Importantly, these bowls may be simplistically viewed as partially filled soup bowls, rather than soup bowls entirely filled. That is, the sample is distributed as a surface bowl-like structure, migration through the radial central region of the column being faster and also more efficient (with respect to reduced plate height) than near the wall. The problem that is therefore faced by the chromatographer is that more theoretical plates are required to separate bowl-like migration profiles than thin flat discs.

This limitation in the chromatographic column could be overcome if the detector were placed in a localised region inside the bed or at localised region at the end of the column inside the frit. In such a location the detector could only see a localised region of the sample band. Hence complete separation need only be achieved within the vicinity of the detector itself, not necessarily across the entire column cross section. As such, fewer theoretical plates would be required to separate just the surfaces of these bowls than the entire bowls. End-column detection, or at least a detection process that emulates ‘end-column’ detection would be extremely valuable, since there would be less reliance placed on the bed within the column tube being homogeneous [11,12]. Even though end-column detection has been shown to be very useful for detailing bed heterogeneity, this type of detection process is not mainstream because it is difficult to implement, especially in high through-put laboratories.

In an attempt to develop a robust end-column localised detection process we recently described a new column technology that effectively isolates mobile phase that elutes from the radial central region

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of the column from that of mobile phase that elutes from the radial peripheral region of the column — i.e., near the wall [13–15]. These columns utilised a new type of multiport end fitting that separately channelled these flow paths out from the column. This multiport end fitting was also fitted with a two-piece annular frit that prevented cross flow between these two regions. This column has been referred to as a parallel segmented flow column. Flow that eluted from the central region was channelled through a detector separately from that of the flow that eluted from the peripheral region. This in effect enabled a bulk detector to treat separately the analysis of sample in either of these zones: Effectively this design emulated end-column detection, but with the bulk flow advantages. A benefit of this column design was that the solute band recovered from the radial central region was more concentrated than the sample otherwise recovered from the bulk sample band using a conventional column [13,14]. The sample band that was extracted from the radial central region of the column was also less dispersed than the bulk sample in its entirety because sample was not diluted by the tailing wall portion of flow. Hence columns fitted with these end fittings operate with more theoretical plates than conventional columns. Reduced plate heights (h) on these columns have been reported as low as 1.67 for fully porous 5 μm particles [14].

While parallel segmented flow columns provide a great advantage in reducing the plate height, gains in sensitivity were increased by relatively modest degrees, typically 20%. In order to improve sensitivity in analysis we also incorporated this new type of fitting at the column inlet; this design was referred to as a curtain flow column [16]. The benefit of using the new multiport fitting with the annular frit at the column inlet as well as at the outlet was that it allowed for separations to occur based on the principles of the 'infinite diameter column', but in a manner that allowed for automated injection and detection [16]. Using this header/frit design allowed for sample to be loaded into the radial central inlet portion of the column, rather than as a central point injection, and this enabled a greater portion of the bed to be utilised. Any radial dispersion to the wall section from the centralised injection is corrected at the point of elution because the column is also fitted with the parallel segmented flow outlet fitting.

A question that was unanswered in our original communication [16] that detailed curtain flow chromatography was how reliable was the process and what were the analytical figures of merit in comparison to chromatography that utilised conventional columns. The purpose of this communication is therefore to detail aspects of quantification in curtain flow chromatography.

2. Experimental

2.1. Chromatography column

Reversed phase Hypersil GOLD chromatography columns were supplied by ThermoFisher Scientific (Runcorn, Cheshire, United Kingdom). In total, two complete sets of columns were tested. Each column in these sets was 50 mm in length. Each set also contained three conventional columns with internal diameters of 4.6 mm, 3.0 mm and 2.1 mm. Each set also contained a curtain flow column with an internal diameter of 4.6 mm.

2.2. Chemicals and reagents

All mobile phases were prepared from HPLC-grade solvents purchased from Merck (Kilsyth, Victoria, Australia) or Fisher Chemicals (Loughborough, UK). All chemicals were commercially available. Phenetole, butylbenzene and pentylbenzene were purchased from Sigma Aldrich. Milli-Q water (18.2 MΩ cm^{-1}) was prepared in-house and filtered through a 0.2 μm filter.

2.3. Reagents

Stock solutions of the standard test mixture were accurately prepared in mobile phases at the concentrations approximately 16.90 mM (phenetole), 29.89 mM (butylbenzene) and 27.81 mM (pentylbenzene). The standard curve was generated using standards prepared by serial dilution.

2.4. Chromatographic separation

Chromatographic experiments were conducted on three instruments:

(1) 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 a Degassex model DG-440 inline degasser unit.
(2) An Agilent 1290 operated as supplied from the manufacturer. For the Agilent and Shimadzu systems curtain flow mobile phase was supplied using a Shimadzu LC-10ADvp HPLC pump fitted with a Degassex model DG-440 inline degasser unit.
(3) An Ultimate 3000 RS system, used as supplied by the manufacturer. The curtain flow mobile phase on this system was delivered using the 3000 RS dual pump solvent delivery system. Each pump on the Ultimate 3000 RS system can be independently controlled, including the delivery of up to four different solvent streams per delivery system. The advantage of this type of solvent delivery system is that it enabled precise control of the flow portions to either the wall or central sections using just the one incorporated delivery system.

All analyses were conducted under isocratic conditions using a mobile phase of 20:80 water: methanol. We tested the performance on three systems independently, at various flow rates. The flow rate through each column was adjusted in accordance with the column internal diameter to maintain constant linear velocity. On the Ultimate 3000 system the flow rates were 1.5 mL/min, 0.64 mL/min and 0.31 mL/min for the 4.6 mm, 3.0 mm and 2.1 mm i.d. columns respectively. While on the Agilent 1290 system the flow rates were 3.0 mL/min 1.28 mL/min and 0.63 mL/min for the 4.6 mm, 3.0 mm and 2.1 mm i.d. columns respectively. Only the 4.6 mm i.d. column was tested on the Shimadzu system and the flow rate was 2.0 mL/min. The injection volumes used were 5 μL for 4.6 mm i.d. columns, 2.12 μL for 3.0 mm i.d. and 1.04 μL for 2.1 mm i.d. columns, in accordance with the comparative column volumes. UV detection was performed at 254 nm. Analysis on the Agilent system was repeated in replicates of at least 5, on the Shimadzu and Ultimate 3000 systems in triplicate.

3. Results and discussion

For a column to function as an 'infinite diameter' column sample must be injected into the radial centre at the inlet. During migration through the bed the sample ideally should not reach the wall. In order for a column to function as an infinite diameter column the sample must enter into the column such that it is not dispersed to the wall. Therefore the sample should not pass through the inlet frit, otherwise it will be immediately dispersed to the wall. Therefore in order for a column to operate as the infinite diameter column the injection needle is typically inserted through the frit and into the bed. However, insertion of a needle into the bed can be destructive, and the process as a whole is not conducive to automated analysis. Furthermore, when sample is introduced in this manner, as a central point, only a limited amount of stationary phase is employed within the column. A large portion of stationary phase is therefore not utilised.