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Characterization of radial and axial heterogeneities of chromatographic columns by flow reversal

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ABSTRACT

The impact of the column length (5, 10, and 15 cm) and packing mode (constant pressure and constant flow rate up to 15,000 psi) on the radial and axial heterogeneities of 3.0 mm i.d. research prototype columns packed with the same batch of 2.4 μm BEH-C_{18} particles was investigated by the flow reversal technique. The data were gathered for a non-retained marker (uracil, acetonitrile/water eluent mixture, 80/20, v/v, flow rate 0.5 mL/min, T = 297 K) and revealed that the radial heterogeneity of the packed bed, characterized by the center-to-wall relative velocity bias (ω_β) and its length scale, is nearly independent on the packing mode: the velocity biases extend over a same length scale estimated at 154 μm while ω_β is in between 4% and 6% for all columns. Secondly, the data revealed that the column length has a slight impact on ω_β: assuming a two-region (wall and center regions) stochastic model of transcolumn eddy dispersion, ω_β increases from 4.6% to 5.1% and to 6.1% for 5, 10, and 15 cm long columns, respectively, when packed at constant flow rate. For columns packed at constant pressure, ω_β increases from 5.0% to 5.2% and to 5.6%, respectively. Finally, it is found that all columns are axially heterogeneous: the bottom half, which is packed first (column inlet), is slightly more uniform than the top half (column outlet) which is packed last. Overall, the results of the flow reversal experiments corroborate recent observations (130 μm thick wall region and ω_β = 5.0%) based on flow simulations in a focused-ion-beam scanning electron microscopy (FIB-SEM) based 3D reconstruction from a 2.1 mm × 50 mm column packed with 2 μm BEH-C_{18} particles.

1. Introduction

It has been massively observed in the past literature that the efficiency of chromatographic columns (from capillary to narrow-bore, analytical, and to preparative-scale format) is severely limited by the flow unevenness across their diameter [1–12]. The existence of a transverse flow heterogeneity is intrinsically related to column packing that involves a large number of experimental parameters such as the nature of the pushing and slurry solvents, the concentration of the slurry solutions, the maximum packing pressure, constant pressure or constant flow rate packing model, the dimensions of the tube containing the slurry solution, the inner surface treatment of the column, radial or axial compression packing, and the application of ultrasonic irradiation [13–15]. As a result, there is no clear understanding of the actual mechanism of bed formation in modern 2.1–4.6 mm i.d. columns. Columns are packed after optimization of the experimental parameters which are specific to the nature of the particles (size, density, chemistry) and to the column dimensions (column-to-particle diameter ratio and column length-to-column diameter ratio). There is no commonly accepted theory of column packing yet available. For that all, some general rules and packing strategies are proposed in the literature [13,14] but they do not apply to all packing materials and column dimensions. In essence, the mechanism of bed formation obeys to the rheology of a thick particle slurry or paste which experiences a complex stress distribution along and across a cylindrical stainless steel tube during a pressure-driven filtration process. Scarce are the relevant research works in this area of chromatographic science [16,17]. Therefore, packing methods and their variables are essentially optimized by trial and error so that the bed remains stable (no bed collapse), durable (repeatable 1000 injections or more at maximum system pressure), and efficient enough for applications in high pressure liquid chromatography (HPLC) and very high pressure liquid chromatography (vHPLC).

Direct structural measurements have unambiguously shown that packed beds are both radially [2,9,18,19] and axially het-

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erogeneous [20,21]. These physical investigations have helped formulating relationships between packing parameters, bed structure, and kinetic performance of chromatographic columns [22,19,23–26,9]. Among others, a recent experimental technique based on flow simulations in a focused-ion-beam scanning electron microscopy (FIB-SEM) based 3D reconstruction from a 2.1 mm × 50 mm column packed with 2 μm BEH-C_{18} particles has revealed the existence of three concentric zones across the packed bed: (1) a thin, loose, and orderly packed region at the wall of the column (a few particle diameters wide), (2) an intermediate thick, dense, and randomly packed layer (130 μm wide) and (3) a bulk, central, and randomly packed region. Fluid-flow simulations then revealed minor radial flow heterogeneities, which are directly responsible for the lower than expected efficiency of modern columns relative to the infinite diameter column [27,28]. The existence of a dense intermediate zone, in which the local velocity can be as low as 75% of the bulk velocity [9], is explained by the higher stress imposed in the wall region than that acting in the bulk region [28]. The loss in column performance is the price to pay for producing stable and durable chromatographic columns. While columns packed with fully porous particles (FPP) can theoretically deliver reduced plate heights (RPH) as small as 0.9 [29], the best observed RPHs usually lie in between 1.7 and 2.0. Remarkably, lower RPHs were recently observed at 1.4 after the introduction of the new sub-3 μm superficially porous particles (SPP). Compared to smooth FPPs, the strain of rough SPPs during bed consolidation does not depend much on the radial position across the column diameter and leads to more radially uniform beds. This explanation corroborates the external porosities of columns packed with FPPs (around 37% [30,31]) and SPPs (around 40% [32–34]) and the observed Kozeny–Carman permeability constants (around 140–170 and 200–240 for FPPs and SPPs, respectively [35,30–34,36]). Ideally, radial structure heterogeneity can be reduced by applying gravity-driven dry packing. Yet, it is only achievable with large non-porous particles (210 μm), large column i.d. (1 cm), and the resulting beds are not stable due to the absence of consolidation under high pressures [37].

Moreover, indirect techniques have been used to assess flow heterogeneities across chromatographic columns. They include microvoltammetry [4,6,8], optical fibers in a fluorescence-detection scheme [5], or X-ray computed tomography [10]. Also, the flow reversal technique has brought some interest for the characterization of structural heterogeneities. Indeed, by reversing the flow direction before the analyte exits the column, it is possible to refocus partially the chromatographic band and to assess flow unevenness and axial dispersion across and along the column, respectively. Kaminski [38] applied flow reversal to preparative liquid chromatography columns of i.d. from 17 to 52 mm and of length from 100 to 450 mm. They concluded that this method allowed for the better characterization of mass dispersion in these columns. Similarly, Roper and Lightfoot [39] used flow reversal in order to eliminate the distortion of the effluent profiles across 5 mm × 17.2 and 12.7 mm i.d. stacked-membranes. They were able to measure the local plate heights in full agreement with dispersive theory. Guo and Frey [40] applied flow reversal to show that transcolumn contribution to convective dispersion was responsible for the limited efficiency of various HPLC columns packed with large particle diameters (10 μm and above). More recently, Felinger et al. [41] used flow reversal to determine the dispersion caused by the inlet and outlet flow distributor/frit assemblies, which affect the performance of modern particulate columns for weakly retained compounds.

In this work, the flow reversal technique is applied to assess the degree of axial and radial heterogeneity of six 3.0 mm i.d. research prototype columns (5, 10, and 15 cm long) packed at either constant pressure (15,000 psi) or constant flow rate (pressure ramp up to 15,000 psi). All the other experimental parameters were kept the same (batch of 2.4 μm BEH-C_{18} FPPs, nature of the pushing and slurry solvents, slurry concentration, and initial packing pressure set at 2000 psi). No further attempts were made to maximize the column performance. The main goal is to investigate the relationship between the packing parameters (3 column lengths, 2 packing modes) and the bed structure. The results of the flow reversal experiments are interpreted from a stochastic model of long-range eddy dispersion [42] coupled to Gidding's general theory of band broadening [43]. The wall-to-center relative velocity bias (ω_β) and its length scale are derived for the six columns tested. Both the radial and axial structure heterogeneities of the columns are assessed and the impact of the column length and packing mode on the results are discussed in depth.

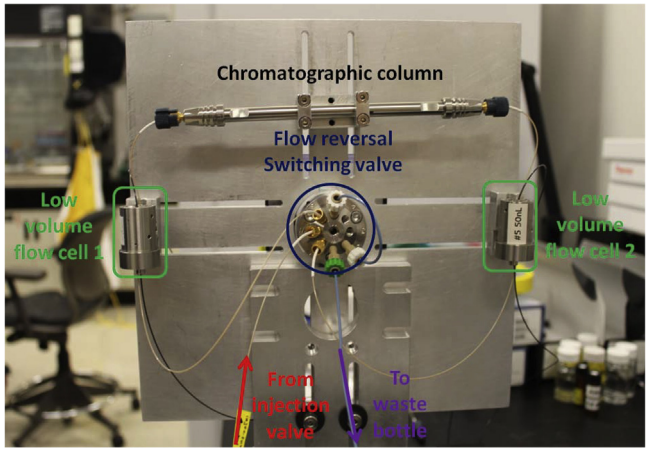

Fig. 1. Experimental set-up used to perform flow reversal experiments based on a two-position switching valve (blue circle) and to record the chromatograms immediately before and after the chromatographic column with two low-volume (50 nL) optical flow cells (green rectangles). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Theory

2.1. Column retention and plate height after an odd and even number of flow reversals

2.1.1. Retention time

The length of the chromatographic column is L. Isocratic conditions are applied, so, the analyte migrates along the column at a constant migration linear velocity u. The initial flow direction is from the inlet to the outlet of the column. When the axial position of the band center reaches an arbitrary position, z_{out}, the flow direction is changed. At that time, the position z_{out} of the analyte is written:

$$z_{out} = (1 - x)L \tag{1}$$

where $0 < x < 0.5$ (see illustration of the flow reversal process in the top graph of Fig. 2).

The analyte is now migrating along the column from the outlet to the inlet of the column. The second flow reversal event occurs when its band center is reaching a new axial position, z_{in}, which is symmetrical to the position $(1 - x)L$ with respect to the middle of the column. The position z_{in} of the analyte zone close to the column inlet is then:

$$z_{in} = xL \tag{2}$$

This succession of forward and backward migration of the analyte is repeated for any number, n, of flow reversals. Accordingly,