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On the relationship between radial structure heterogeneities and efficiency of chromatographic columns

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ABSTRACT

The general dispersion theory of Aris is applied to predict the virtual asymptotic dispersion behavior of packed columns. The derived model is also used to estimate the actual pre-asymptotic dispersion behavior of modern 2.1 mm × 50 mm columns packed with sub-2 μm fully porous particles (FPPs) during the transient dispersion regime. The model accounts for the actual radial distribution of the flow velocity across the column diameter. From the wall to the center of the column, focused-ion-beam scanning electron microscopy (FIB-SEM) experiments were recently performed to reveal the existence of a thin ($0.15d_p$ wide, d_p is the average particle diameter) hydrodynamic boundary layer (THBL), a thin ($3d_p$ wide) and loose orderly packed layer (TLOPL), a $60d_p$ wide and dense randomly packed layer (WDRPL), and a large ($\simeq 460d_p$) randomly packed bulk central region [1].

The theoretical calculations of the actual pre-asymptotic reduced van Deemter curves (2.1 mm × 50 mm column, sub-2 μm BEH-C_{18} FPPs, *n*-hexanophenone analyte, acetonitrile/water eluent, 80/20, v/v, flow rate from 0.05 to 0.35 mL/min) confirm that the impact of the sole THBL on column dispersion can be neglected. In contrast, the contribution of the TLOPL to the reduced plate height (RPH) is about 0.2 h unit at optimum reduced velocity. Most remarkably, the negative impact of the TLOPL on column performance may be fully compensated by the presence of the adjacent WDRPL if the depth of the velocity well were to be 5% of the bulk velocity. In actual 2.1 mm × 50 mm columns packed with sub-2 μm FPPs, this velocity depth is as large as 25% of the bulk velocity causing a significant RPH deviation of 0.7 h unit from the RPH of the bulk packing free from wall effects. Maximum column performance is expected for a reduction of WDRPL density. This suggests optimizing the packing process by finding the proper balance between the stress gradient across the WDRPL (responsible for the deep velocity well) and the friction forces between the packed particles (responsible for the rearrangement of the particles during bed consolidation). Past and recently reported RPH data support the theoretical insights: the stress gradient/particle friction balance in the WDRPL is better realized when packing superficially porous particles (SPPs) rather than FPPs in 2.1–4.6 mm i.d. columns (the RPH deviation is reduced to 0.4 h unit) or sub-2 μm particles in 100 cm × 75 μm i.d. capillaries combining high slurry concentrations and sonication (the RPH deviation is reduced to only 0.15 h unit).

1. Introduction

The performance of modern narrow-bore (2.1 mm i.d.) ultrahigh-pressure liquid chromatography (UHPLC) columns packed with either sub-2 μm FPPs or sub-2 μm SPPs is limited by the presence of the column hardware embedding the packed particles [2–4]. While the expected minimum RPH related to axial dispersion along random sphere packings is expected to be in the range from 0.5 for non-porous particles (NPPs) to 0.9 for FPPs in the absence of geometrical confinement [5,6], the observed minimum RPHs are typically 1.4 and 2.0 for commercial UHPLC columns optimally packed with SPPs and FPPs, respectively [7–9]. On one hand, the presence of the frits, the inlet flow distributor and of the outlet flow distributor may affect the performance of short columns (< 5 cm long) when poorly retained compounds are injected [10]. On the other hand, during the packing process, the presence of the stationary wall of the column (stainless steel tube for particulate columns) causes a higher stress in the peripheral region than that in the central bulk region [11,12]. This stress gradient induces density gradients in the bed and non-uniform flow profiles across the column diameter. Additionally, the flat surface of the column wall forces a two-dimensional geometrical arrangement of the spher-

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ical particles *versus* a three-dimensional packing arrangement in the bulk region. This structural heterogeneity applies to any column irrespective of their length and inner diameter unless the ratio of the inner column diameter to the particle diameter falls below 7 [13–15]. For such small column to particle diameter ratio, the geometrical wall constraint extends to the entire column diameter and the bed structure is found radially uniform [15]. For column aspect ratios larger than 7, the bed structure is no longer uniform across the entire column diameter: serious velocity disturbances are expected in the wall region relative to those across the uniform bed structure in the central region. These trans-column velocity biases may seriously affect the performance and resolution power of UHPLC columns [16–18].

Indirect experimental evidences of trans-column velocity biases and structural heterogeneity of the packed bed have been reported in the past literature. This was achieved for wide i.d. columns with microvoltammetric electrodes [19–21], optical fibers in a fluorescence-detection scheme [22], flow reversal [23] or with X-ray computed tomography [24], among others. Very recently, these observations were refined for narrow-bore UHPLC columns (2.1 mm × 50 mm) packed with sub-2 μm particles using FIB-SEM followed by image processing and fluid-flow simulation [1]. The observed radial distribution of the flow velocity revealed four distinct concentric zones of the packed bed from the wall to the center of the column: (1) an extremely thin ($0.15d_p$ wide, d_p is the particle diameter) hydrodynamic boundary layer (THBL); (2) a thin ($3d_p$ wide) loose and orderly packed layer (TLOPL); (3) a wide ($60d_p$) dense and randomly packed layer (WDRPL); and (4) a large ($460d_p$) central bulk region. The local velocity increases from 0 to $2.2u_{bulk}$ (u_{bulk} is the average velocity in the bulk region) across the THBL, oscillates and decreases from $2.2u_{bulk}$ to $0.75u_{bulk}$ in the TLOPL, increases from $0.75u_{bulk}$ to u_{bulk} across the WDRPL and remains uniform at the bulk velocity across the entire central region. Active flow technology (AFT) has helped solving this problem of transcolumn flow heterogeneity. Shalliker and co-workers have amply proven the benefit of AFT on column performance [25–29] while others independently confirmed this advantage [30–32]. Regrettably, this approach remains impractical because it requires using proper outlet restrictors to optimize the flow rate ratio between the wall and the center region of the column. Additionally, the optimum flow rate ratio is somewhat dependent on column length, column diameter, retention mode, and on the nature of the small analyte. Therefore, progress in column performance is better linked to the development of more uniformly packed columns, ordered structures [33], or of 3D printing technologies [34] than it is to AFT. The unprecedented work and achievement made by Reising and Tallarek regarding the complete structural heterogeneities of the packed bed across a narrow-bore UHPLC column have opened new fundamental and practical roads towards the complete understanding of the complex relationship between bed structure and column performance. Ultimately, this work should guide the practitioners towards the proper development and optimization of packing procedures.

In this work, on the basis of the accurate radial distribution of the fluid-flow velocity observed across 2.1 mm i.d. UHPLC columns [1], the impacts of the radial structure heterogeneities of packed beds (including the THBL, the TLOPL, the WDRPL, and the central bulk region) on the performance of narrow-bore UHPLC columns is investigated in depth. First, the exact expression of the asymptotic (long-time) dispersion coefficient along short 5 cm long UHPLC columns is derived by extending the applications of the Aris general dispersion theory [35] from open coaxial cylinders to filled packed columns. It is important to keep in mind that the present theoretical development aims at studying the impact of the structural heterogeneities of columns on column efficiency. By no means is it about investigating the fundamental impact of thermal effects and radial temperature gradients on column performance when operating UHPLC columns at very high pressures (>10 000 psi) and high flow rates (> 1 mL/min). This problem was tackled in depth during the 2000s when UHPLC technology was emerging [36–41]. Secondly, the relationship between the long-time asymptotic RPHs (Aris) and the observed pre-asymptotic RPHs is empirically determined from the study of the transient dispersion behavior of a small molecule (*n*-hexanophenone) on a 2.1 mm × 50 mm column packed with sub-2 μm BEH-C_{18} particles. Thirdly, the application of Aris dispersion theory enables to evaluate the impact of the combined existence of four different fluid-flow velocity regions on column performance for various and arbitrarily designed radial velocity profiles. The results are reported and discussed in terms of the structure–performance relationship for a chromatographic column. Finally, solutions are proposed to minimize the performance losses of narrow-bore columns packed with sub-2 μm particles by acting properly on the selection of the packing process conditions and on the morphology of the particles to be packed.

2. Theory

The first part of this section summarizes the main results derived by Aris in 1959 for the prediction of the asymptotic axial dispersion coefficient of analytes by convection, diffusion, and exchange between two cylindrical and coaxial phases [35]. These results are valid for infinitely long columns or at infinitely long times, e.g., when the concentration distributions tend towards a symmetrical Gaussian distribution [42]. In the second part, the formalism of Aris is directly applied to the determination of the asymptotic dispersion coefficients of analytes along UHPLC narrow-bore columns packed with sub-2 μm spherical particles. The fundamental equations enable the calculation of the asymptotic trans-column eddy dispersion RPH from the actual distribution of the fluid-flow velocity recently obtained by flow simulations in a FIB-SEM based 3D reconstruction from a 2.1 mm × 50 mm column packed with sub-2 μm BEH-C_{18} particles [1].

2.1. Aris general theory of analyte dispersion by convection, diffusion, and exchange between phases

Aris rigorously derived the increase rate of the spatial peak variance, $d\mu'_{2,z}/dt$, of the concentration zone migrating along an open tube filled with two coaxial annular phases in contact. Phase 1 is located in between the radial coordinates r_0 and r_1, phase 2 in between radial coordinates r_1 and r_2 and the volume embedded in between the radial coordinates $r=0$ and r_0 is impermeable to the analyte. At equilibrium, the concentrations, c_1 and c_2, of the analyte in phases 1 and 2, respectively, are linked by the equilibrium constant α ($c_2=\alpha c_1$). When the two phases are not in thermodynamic equilibrium, the mass transfer between them is assumed to be given by the linear driving force model [43] in phase 2: the mass flux is then $k(c_2 - kc_1)$, where k is the rate constant (see Fig. 1). The mathematical formalism of Aris provides the time-independent expression of $d\mu'_{2,z}/dt$ under asymptotic conditions. This expression is given by [35]:

$$\frac{1}{2}\frac{d\mu'_{2,z}}{dt} = \beta\left(D_1 + \kappa_1\frac{U_1^2[r_1^2 - r_0^2]}{D_1}\right) + (1-\beta)\left(D_2 + \kappa_2\frac{U_2^2[r_2^2 - r_1^2]}{D_2}\right) + \frac{\left(s\beta[1-\beta][U_1 - U_2]\right)^2}{2k\alpha r_1} \quad (1)$$

where $s = \sqrt{(r_1^2 - r_0^2) + \alpha(r_2^2 - r_1^2)}$ and $\beta = r_1^2 - r_0^2/s^2$.