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Larger voids in mechanically stable, loose packings of 1.3 μm frictional, cohesive particles: Their reconstruction, statistical analysis, and impact on separation efficiency

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ABSTRACT

Lateral transcolumn heterogeneities and the presence of larger voids in a packing (comparable to the particle size) can limit the preparation of efficient chromatographic columns. Optimizing and understanding the packing process provides keys to better packing structures and column performance. Here, we investigate the slurry-packing process for a set of capillary columns packed with C18-modified, 1.3 μm bridged-ethyl hybrid porous silica particles. The slurry concentration used for packing 75 μm i.d. fused-silica capillaries was increased gradually from 5 to 50 mg/mL. An intermediate concentration (20 mg/mL) resulted in the best separation efficiency. Three capillaries from the set representing low, intermediate, and high slurry concentrations were further used for three-dimensional bed reconstruction by confocal laser scanning microscopy and morphological analysis of the bed structure. Previous studies suggest increased slurry concentrations will result in higher column efficiency due to the suppression of transcolumn bed heterogeneities, but only up to a critical concentration. Too concentrated slurries favour the formation of larger packing voids (reaching the size of the average particle diameter). Especially large voids, which can accommodate particles from >90% of the particle size distribution, are responsible for a decrease in column efficiency at high slurry concentrations. Our work illuminates the increasing difficulty of achieving high bed densities with small, frictional, cohesive particles. As particle size decreases interparticle forces become increasingly important and hinder the ease of particle sliding during column packing. While an optimal slurry concentration is identified with respect to bed morphology and separation efficiency under conditions in this work, our results suggest adjustments of this concentration are required with regard to particle size, surface roughness, column dimensions, slurry liquid, and external effects utilized during the packing process (pressure protocol, ultrasound, electric fields).

1. Introduction

The reduction of the mean particle size in packed columns is a consistent goal in liquid chromatography because the minimum plate height and overall column efficiency (plates per meter) scale with the particle diameter [1] (as long as the packing structure and bed homogeneity are conserved independently from the particle size). For example, capillary columns packed with sub-2 μm particles achieve theoretical plate numbers of ~500,000 per meter, with peak capacities up to 1500 [1,2]. They are of special interest in separations of complex biological samples, where high resolution between analytes is desired before detection. Nevertheless, the use of ever smaller particles amplifies several problems, including extra-column band broadening [3], frictional heating [4–6], back-pressure [7], and radial expansion of the column under high pressure [8]. Further, the reduced particle diameter and the increased back-pressure present major challenges to the formation of a uniform bed structure, with a major contribution to the packed bed's separation efficiency originating from transcolumn heterogeneities introduced during the packing process [7,9]. Gritti and Guiochon [10] estimated that transcolumn dispersion induced by heterogeneities across the column diameter makes up to 70% of the total dispersion for analytical ultrahigh-pressure liquid chromatography (UHPLC) columns; differences in bed morphology between

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the column wall region and the bulk packed bed are a main factor here [5,11–15].

The work of Shalliker et al [12]. which holds evidence for two different wall effects in chromatographic columns, describes the situation well. The geometrical wall effect caused by the first (~5) layers of particles adjacent to the wall is characterized by higher than average external porosity due to ordered packing, since the particles can only touch the wall but not penetrate it. The first particle layer at the wall is a highly ordered monolayer, followed by more imperfect layers with decreasing order until a random particle arrangement is reached. As a result, the local external porosity directly at the column wall tends towards unity, reaches a minimum after approximately a particle radius from the wall, and oscillates over a few particle diameters (d_p) towards the average value found in the column center (bulk packing region). This packing induces an oscillating radial flow velocity profile in this region [14], with locally higher velocities at radial positions where porosity is increased. This results in an overall higher average flow velocity in this wall region than in the bulk packing region. These macroscopic (wall-to-wall) morphological and velocity heterogeneities result in transcolumn dispersion [13–15]. The second wall effect begins at a radial coordinate >5 d_p from the wall and is due to radial stress exerting forces on the particles against the wall and the friction between the bed and the column wall during the packing process [12,16]. It results in a denser packed intermediate region of ~50 d_p from the column wall towards the column center. Since the capillary columns in the current work have a diameter of ~58 d_p (column inner diameter: 75 μm; average particle diameter, d_p = 1.3 μm), their transcolumn bed morphology is affected mostly by the geometrical wall effect.

The formation of a homogeneous bed from pore to transcolumn scale is a key behind efficient chromatography. It was only during the last years that the search for the optimal packing conditions started to be based on a more detailed understanding of the process itself [12,17–23], in addition to purely empirical optimization. Many parameters influence the packing process and final bed structure. Recent work from our groups addressed experimentally the effects of particle properties such as the width of the particle size distribution (PSD) and the surface roughness [24], different capillary column diameters [25], and conduit geometry in HPLC microchips [26,27] on separation efficiency. Importantly, the chromatographic performance of these packed columns was complemented with the three-dimensional (3D) physical reconstruction [24,25] or simulation [13–15,27] of packing microstructures followed by a detailed analysis of relevant morphological features and resulting (experimental and/or simulated) transport properties.

Nowadays, a portfolio of techniques is available for detailed investigation of the 3D morphology in macroporous–mesoporous chromatographic supports such as packed beds of small mesoporous particles or silica-based and polymeric monoliths. This portfolio [28–34] includes confocal laser scanning microscopy (CLSM) [35], focused ion-beam scanning electron microscopy (FIB-SEM) [36], and serial block-face scanning SEM (SBF-SEM) [37] for the reconstruction of the interstitial macropore space in packed beds [24,25,38] and monoliths [39–44], and scanning transission electron microscopy (STEM) [45,46] for the reconstruction of the intraparticle or intraskeleton mesopore space [47–50].

Our recent work on establishing morphology–transport relationships for supports used in liquid chromatography has elucidated, for example, the sample volume that actually needs to be reconstructed for a meaningful morphological description [51], has resolved systematic radial variations in the macropore space morphology of packed columns and monoliths to correlate them with particle properties [24,25] or the monolith preparation protocol [43,52], has quantified heterogeneity length scales and structural correlations in these materials [52,53], or has analyzed geometrical and topological parameters in silica monoliths to detect changes as their domain size is reduced to submicrometer dimension [52,54,55]. For packed capillary columns, our previous work [24,25] has correlated experimentally observed differences in column efficiency to particle size segregation and bed structural changes in the wall region (mostly, average packing density). Assuming particle properties including surface roughness or the PSD are insignificant, column efficiency is dominated by transcolumn dispersion, i.e., by how well the critical issue of the wall region (local bed density and homogeneity governing fluid flow and dispersion) has been resolved with respect to the bulk packing structure.

In a subsequent study [56], we noticed the importance of the slurry concentration (regarding the final bed morphology) in column packing for three sets of columns, each including one column packed with a low slurry concentration and one packed with a higher concentration. The column packed with a higher concentration showed better efficiency for all three types of particles, i.e., fully porous 1.7 and 1.9 μm bridged-ethyl hybrid (BEH) particles and 1.9 μm Kinetex core–shell particles. We observed that the BEH particles (with a relative standard deviation of their PSD of 12–16%) favoured size segregation at low slurry concentrations, while it was suppressed at higher concentrations. Further, with high slurry concentrations, there was a clear increase in the number of larger voids in the bed structure, which can have a dramatic effect on column efficiency, as illustrated by Schure and Maier [57] with dispersion simulations in defective packings. They concluded that it is far more important in column packing to prevent defect sites, e.g., larger voids or even gaps, leading to inhomogeneous packing rather than obtaining the highest packing density. For core-shell particles (Kinetex), a slight densification in the wall region was observed at higher slurry concentration, but the number of larger voids showed only a very small increase [56]. Based on these observations, we proposed the operation of two antagonizing effects as the slurry concentration is increased from low to high values: the first one reduces wall effects at higher slurry concentration, which reduces transcolumn bed heterogeneities and associated dispersion; the second one increases the number of larger voids in the bed, which increases chromatographic band broadening. This suggests an intermediate slurry concentration would result in the best performance, that is, with only weak wall effects and yet no critical amount of larger voids.

To test this hypothesis we prepared a set of capillary columns with a series of increasing slurry concentrations. Nine columns having 75 μm i.d. were packed using fully porous 1.3 μm C18 BEH particles at slurry concentrations from 5 to 50 mg/mL. Each column was characterized regarding separation efficiency and three capillary columns representing low, intermediate, and high slurry concentrations were reconstructed three-dimensionally using CLSM for analysis of their efficiency limiting morphological features. The analysis focused on transcolumn heterogeneities as well as the occurrence of larger voids in the bed structure. The purpose of this work is not to conclude definitively on the absolute effect of slurry concentration on bed morphologies. Instead we seek to give description of morphological heterogeneities formed as a function of the slurry concentration, with general implications for column packing.

2. Experimental

2.1. Chemicals and materials

75 μm i.d. cylindrical fused-silica tubing was purchased from Polymicro Technologies (Phoenix, AZ). The capillaries were packed