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Short communication

Implementation of high slurry concentration and sonication to pack high-efficiency, meter-long capillary ultrahigh pressure liquid chromatography columns

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A B S T R A C T

Slurry packing capillary columns for ultrahigh pressure liquid chromatography is complicated by many interdependent experimental variables. Previous results have suggested that combination of high slurry concentration and sonication during packing would create homogeneous bed microstructures and yield highly efficient capillary columns. Herein, the effect of sonication while packing very high slurry concentrations is presented. A series of six, 1 m × 75 μm internal diameter columns were packed with 200 mg/mL slurries of 2.02 μm bridged-ethyl hybrid silica particles. Three of the columns underwent sonication during packing and yielded highly efficient separations with reduced plate heights as low as 1.05.

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1. Introduction

The benefits of sub-2 μm chromatographic supports have largely focused separation technologies towards ultrahigh pressure liquid chromatography (UHPLC) [1]. Theoretically, sorbents on this scale provide higher separation efficiencies and shorter separation times. True benefit from these materials is dependent, however, on homogeneous packing of the sorbent into a column. This is complicated by the smaller particle's requirement of significantly increased packing pressure and presents a major challenge in the creation of a uniform bed structure. As packing material continues in the direction of smaller particles, dispersion due to transcolumn heterogeneity becomes significantly more important. This type of dispersion is estimated to account for up to 70% of the total dispersion for small molecules in UHPLC columns [2].

Packing that results in a well performing column requires the formation of a homogeneous bed structure across all scales, from transchannel to transcolumn, within the column [3]. Study of "optimal" packing conditions has lead to more detailed understanding of the physical process [4–14]. Unfortunately this process is dynamic and highly influenced by many interdependent parameters. The results of these studies have yielded many opinions on the "art" of column packing as opposed to the science. More recently, collaboration between our groups has examined certain packing parameters including the effects of particle properties, capillary column diameter and slurry concentration [15–17]. Most importantly these studies have been informed by three-dimensional reconstructions via confocal laser scanning microscopy (CLSM) of the packing microstructure. These renderings have allowed for detailed expositions of morphological features corresponding to specific packing conditions, which are then related to explain the column's kinetic performance.

Morphological heterogeneity between a column's wall region and bulk packing is often the main contributor to poor column performance [18–23]. Previous studies have indicated that the differences in these regions are dependent on slurry concentration [16,17]. Detailed understanding of the packing microstructure has guided empirical packing studies to obtain well performing columns. For example, our initial results suggest that there is a specific "intermediate" slurry concentration capable of balancing the antagonizing effects associated with a low or high slurry concentration for each particle diameter [16]. Further study of this proposition confirmed balancing of packing defects and demonstrated that increasing slurry concentration suppresses wall effects

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and reduces transcolumn bed heterogeneities through prevention of both locally high porosity and particle size segregation [17].

We further noticed that even though slurry concentrations higher than "optimal" continue to suppress wall effects, the columns perform poorly [17]. The benefits of high slurry concentrations eventually begin to diminish as the number and size of packing voids in the bed structure increases. Giddings suggested voids would impact eddy dispersion negatively and contribute significantly to chromatographic band broadening [3]. Voids increase velocity extremes and eddy dispersion on both transchannel and short-range interchannel scales. The detriment of incorporated voids was also illustrated with dispersion simulations that resulted in stating the column's overall performance is more dependent on reduction of large voids than obtaining high packing densities [24].

The conclusions to our most recent study propose that even higher efficiency UHPLC columns may result from formation of a homogenous bed structure across the entire column through the combination of high slurry concentration and sonication to prevent the formation of larger voids [17]. To date sonication has been used in column packing, but only to limited effect and not in association with very high slurry concentrations [25–29]. Reduction of the total number of voids should allow for realization of more homogeneous and highly efficient columns.

To test this a set of 6 capillary columns, all 1 m in length × 75 μm internal diameter (i.d.), were packed with 200 mg/mL slurries. Three of the columns were packed with the application of sonication and three were not. Columns packed with sonication yielded performance with reduced plate heights approaching 1 and a realized (instead of extrapolated as often reported) 470,000 plates/m.

2. Materials and methods

2.1. Chemicals and materials

75 μm i.d. cylindrical fused-silica tubing was purchased from Polymicro Technologies (Phoenix, AZ). The capillaries were packed with C18-modified bridged-ethyl hybrid (BEH) silica particles provided by Waters Corporation (Milford, MA) with a Sauter diameter of 2.02 μm. The Sauter diameter was calculated from a scanning electron microscope (SEM) based particle size distribution obtained from the measurement of ∼1200 C18-modified 1.9 μm BEH particles from the same batch using a JSM-7500F SEM (Joel, München, Germany). HPLC grade acetonitrile, acetone (reagent grade), trifluoroacetic acid (TFA), and the test analytes for chromatographic characterization (L-ascorbic acid, hydroquinone, resorcinol, catechol, 4-methyl catechol) were obtained from Fisher Scientific (St. Louis, MO). Kasil frits for the packed capillaries were prepared with potassium silicate from PQ Corporation (Valley Forge, PA) and formamide from Sigma–Aldrich (St. Louis, MO). HPLC grade water for chromatographic experiments was obtained from a Millipore NANOpure water system (Billerica, MA).

2.2. Preparation of capillary UHPLC columns

Preparation of the capillary UHPLC columns has been described previously in detail [8–14]. Modifications to the procedure will be highlighted here. Column blanks (160 cm × 75 μm i.d.) were fritted using the Kasil method [30]. The extra 60 cm was needed to over pack slightly to allow for bed compression (∼10 cm) as well as to reach from the packing vessel to the sonication bath (∼50 cm). In order to prepare outlet frits, the ends of capillaries were depressed onto a glass microfiber filter (Reeve Angel, Clifton, NJ) wetted with 50/50 (v/v) potassium silicate/formamide. The column blanks were then dried overnight at 50 °C and the resulting frits were ∼125 μm in length. Slurries were prepared by mixing a known mass of the particles in a known volume of acetone (to achieve 200 mg/mL) and suspended with a 10 min sonication cycle using a Cole Parmer Ultrasonic Cleaner 8891 (Vernon Hills, IL).

Prior to packing, the inlet to the column blank was fixed within a UHPLC fitting. The outlet was threaded through the top of a piece of shipping foam padding that was cut to fit snugly within the sonication bath's included basket. The portion of capillary blank to be packed (in this case ∼108 cm) was pulled through the top of the foam entirely. This portion of the column blank was then coiled and taped to the bottom of the foam padding to keep it in place. To ensure the created outlet frit did not loose integrity due to sonication, it was threaded back through the shipping foam padding (from the bottom side, in which the majority of the capillary was taped) until the frit and 2 cm of outlet end of the column blank protruded from the top of the foam padding. This arrangement corresponded to the outlet of column blank being 2 cm above the water line in the sonication bath. The slurry was then placed into a packing reservoir and the inlet of the column blank was secured to the reservoir using the already affixed UHPLC fitting. The foam supporting the coiled capillary was placed into the sonication bath, ensuring that the desired final length (already coiled and secured to the bottom of the foam) remained submerged under water and that the 2 cm of the blanks outlet, including the installed frit, remained above the water line. Sonication during packing was conducted with an Elmasonic P 60 H (Elma Schmidbauer GmbH, Singen, Germany) sonication bath. The sonication bath was set to sweep mode at 80 kHz. Packing was initiated using acetone as a pushing solvent at 150 bar from a DSHF-300 Haskel pump (Burbank, CA). The packing pressure was immediately increased to 2070 bar when the 2 visible cm of bed had been packed. The maximum packing pressure was chosen to maintain consistency between these experiments and previously reported packing studies [15–17]. The column was allowed to pack until the formed bed was visible outside the packing foam, which meant the 108 cm of bed had been packed. The temperature of the bath was kept at 30° C by adding a small amount of ice as necessary and measured using the sonication bath's temperature readout on the display. After the desired length was reached, the packing pressure was slowly released to atmospheric pressure. The column was then connected to a DSXHF-903 Haskel pump (Burbank, CA) using an UHPLC injection apparatus. Each column was flushed for 1 h in 50/50 (v/v) water/acetonitrile with 0.1% TFA at 3500 bar, after which the pressure was gradually released and reinitiated at 700 bar to form a temporary inlet frit with a heated wire stripper from Teledyne Interconnect Devices (San Diego, CA). Columns were then clipped to a 100 cm bed length and an inlet frit was installed using the Kasil method.

2.3. Chromatographic analysis

Column efficiency was tested under isocratic elution conditions using a 200 μM test mixture (L-ascorbic acid, dead-time marker; hydroquinone, resorcinol, catechol, and 4-methyl catechol) and an UHPLC injection apparatus [9]. The mobile phase used for evaluation was 50/50 (v/v) water/acetonitrile with 0.1% TFA. Analytes were detected amperometrically. Electrochemical detection was conducted at a 8 μm × 300 μm carbon fiber microelectrode held at +1.1 V vs. Ag/AgCl reference electrode [31]. This electrode was placed at the outlet of the UHPLC column. Current-to-voltage conversion was conducted using an SR750 current amplifier (Stanford Research Systems, Sunnyvale, CA) with a 109 V/A gain and a 3 Hz, 3 dB low-pass bandwidth filter. An Intel Core 2 Duo desktop computer with a 16-bit A/D converter was used to acquire data at 21 Hz. Data were collected with a custom-written LabView 6.0 program (National Instruments, Austin, TX).

Columns were analyzed over a range of mobile phase velocities to create plots of the plate height H vs. the average mobile

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