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Short communication

Highly efficient capillary columns packed with superficially porous particles via sequential column packing

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ABSTRACT

Highly efficient capillary columns packed with superficially porous particles were created for use in ultrahigh pressure liquid chromatography. Superficially porous particles around 1.5 μm in diameter were packed into fused silica capillary columns with 30, 50, and 75 μm internal diameters. To create the columns, several capillary columns were serially packed from the same slurry, with packing progress plots being generated to follow the packing of each column. Characterization of these columns using hydroquinone yielded calculated minimum reduced plate heights as low as 1.24 for the most efficient 30 μm internal diameter column, corresponding to over 500,000 plates/m. At least one highly efficient column (minimum reduced plate height less than 2) was created for all three of the investigated column inner diameters, with the smallest diameter columns having the highest efficiency. This study proves that highly efficient capillary columns can be created using superficially porous particles and shows the efficiency potential of these particles.

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1. Introduction

Superficially porous particles (SPP), often referred to as core–shell particles, have received sustained attention as a particle architecture capable of achieving high separation efficiencies. The best performing analytical millimeter bore superficially porous columns to date have minimum reduced plate heights below 1.5 [1,2]. Following the general trend seen in liquid chromatography, there has been a movement toward smaller particles, with many vendors offering superficially porous particles below 2 μm in diameter [3–5]. When packed in analytical bore columns, however, it has been seen that for superficially porous particles column performance decreases when going to smaller internal diameter (ID), from 4.6 to 2.1 mm [6,7].

As recently reported in a review by Hayes et al. [8], there have been few studies published on capillary columns packed with superficially porous particles. One report by Fanali et al. compares the application of the columns in capillary electrochromatography (CEC) and capillary liquid chromatography [9]. Two studies by Zhang et al. and Roth et al. reported gradient separations of intact protein mixtures using 5 μm superficially porous particles in capillary columns [10,11]. In another report by Fanali et al., 2.6 μm particles packed into capillaries achieved minimum reduced plate heights of 2.5, 2.8, and 4.0 in a 25, 50, and 75 μm ID capillaries, respectively [12]. Other reports by Bruns et al. have shown a best h_{min} value of 2.2 for 2.5 μm particles packed into 100 μm ID capillaries and an h_{min} of 2.4 for a 50 μm ID column packed with 1.9 μm particles [13,14]. As stated in a 2012 paper by Bruns et al., “Thus it seems unsurprising that we failed to pack a highly efficient column with core–shell particles up to this point; also, we are unaware of any work that claims this achievement” [13]. Recently published work from Blue and Jorgenson demonstrated a reduced minimum plate height around 2.4 for 1.1 μm in-house superficially porous particles and below 1.9 for 2.7 μm commercial superficially porous particles, both packed into 30 μm ID capillaries [15].

The current work presents the creation of highly efficient capillary columns packed with superficially porous particles. The motivation for this work is to extend the efficiency advantages seen with superficially porous particles packed into 4.6 mm bore columns to columns of capillary dimension. Moving from millimeter column diameter to capillary column diameter allows the advantages of superficially porous particles to be extended to the application space of nano-LC. To determine the best packing conditions for the columns, differing slurry concentrations and capillary internal diameters were investigated. Previous work has shown that changing slurry concentration [14,15] and capillary column internal diameter [12,16–18] can have an impact on column performance.

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2. Materials/methods

2.1. Materials

For this study, prototype superficially porous particles bonded with C18 were provided by Waters Corporation (Milford, MA). These particles had a coulter average (50%, volume) diameter of 1.45 μm, specific surface area of 88 m^2/g (unbonded), 84 Å pores (unbonded), and a core diameter to particle diameter ratio of 0.76 (data courtesy: Waters Corporation). The particles were used as provided. In-house particle size characterization was done using a Hitachi S-4700 cold cathode field emission scanning electron microscope (SEM; Tokyo, Japan). This yielded a particle diameter of 1.54 μm (N = 108, number average, 4.2% RSD), which was used for calculating reduced parameters. A representative SEM image of these particles is included in the Supplementary Material, Appendix A.

Acetone (HPLC Grade, submicron filtered), acetonitrile (HPLC Grade), and L-ascorbic acid were purchased from Fisher Scientific (Fair Lawn, NJ). Trifluoroacetic acid (TFA), hydroquinone, resorcinol, catechol, 4-methyl catechol, and formamide were purchased from Sigma–Aldrich (St. Louis, MO). A Barnstead NANO pure system from Thermo Scientific (Waltham, MA) was used to purify water. 360 μm outer diameter (OD) fused silica capillary was purchased from Polymicro Technologies (Phoenix, AZ) with nominal internal diameters of 30, 50, and 75 μm. Potassium silicate (Kasil) was purchased from PQ Corporation (Valley Forge, PA). Glass fiber filters were purchased from Whatman (Pittsburgh, PA).

2.2. Capillary column preparation

For this study, capillary columns were prepared with internal diameters (ID) of 30, 50, and 75 μm. The capillary columns were prepared generally following the previously described methodology [14]. For the 30 μm ID columns, the outlet frit was created by pushing a 50-100 μm plug of 2.5 μm nonporous silica particles (Bangs Laboratories, Fishers, IN) 100-200 μm into the capillary and sintering them in place using an electric arcing device [19] to allow for electrochemical detection with a 100-200 μm long carbon fiber microelectrode pushed up to the outlet frit inside the capillary to ensure negligible post column band broadening [18]. For the 50 and 75 μm ID columns, the column outlet was fritted by pushing the outlet repeatedly against a glass microfiber filter wetted with 1:1 v/v potassium silicate/formamide and then heating the column at 50 °C overnight [20]. The detection microelectrode is pushed up to the outlet frits on the 50 and 75 μm ID capillaries to ensure negligible post column band broadening.

Column packing was similar to the high pressure slurry packing of capillary columns that has been described previously [14,17,21]. Briefly, particles were slurried in 0.2 μm filtered acetone and were dispersed using an Elma (Singen, Germany) P30H sonicator at 80 kHz and 100% power in pulse mode for 5-8 min. A new high pressure packing reservoir holding approximately 0.6 mL of liquid, similar to previous designs, was employed in this study. In practice, only about 0.5 mL slurry can be added to the reservoir. The suspended slurry was added to the high pressure packing reservoir and a fritted empty capillary was attached to the reservoir using a high pressure fitting. The slurry was stirred continuously in the packing reservoir using a small stirbar and a Fisher Scientific (Fair Lawn, NJ) magnetic stirplate. The bed was formed by applying pressure from a Haskel (Burbank, CA) DSHF-300 pneumatic amplifier pump with the pushing solvent being acetone. Packing was initiated at the lowest pressure practical then quickly ramped to 30,000 psi. Pump liquid pressure was calculated using the applied air pressure multiplied by the pump's amplification ratio. Column packing progress was followed by use of back illumination with a white LED light to illuminate the bed as it formed. It was found that column packing could be modeled as a square root relation between column length and packing time. A discussion and modeling of this phenomenon is included in the Supplementary Material, Appendix B. After attaining the desired length, the pressure was removed and the column was vented to ambient pressure. The column was then removed and within a few minutes a new unpacked, fritted capillary was attached to the reservoir with the same slurry. Packing was performed with this and subsequent capillaries without any further modification of the slurry.

After packing, the columns were flushed with 60/40 v/v water/acetonitrile + 0.1% TFA using a Haskel (Burbank, CA) DSXHF-903 pneumatic amplifier pump attached to a lab-built high pressure injector (Supplementary Material, Appendix C). The pressure was increased to just over 40,000 psi and held for 1 h. The column was then allowed to slowly depressurize for 1 h, after which time pressure was increased back to 10,000 psi and a temporary frit was created using a heated wire stripper (Teledyne Interconnect Devices, San Diego, CA). After fully depressurizing, the column was removed. The temporary heat frit was then cut off and a glass fiber/potassium silicate/formamide frit was applied, set in place using an electric arcing device [19]. Most columns in this study were between 26 and 33 cm long. Detailed data for each column are provided in the Supplementary Material, Appendix D.

2.3. Capillary column characterization

Isocratic chromatographic characterization in our lab has been reported previously [14,17,21]. For chromatographic evaluation, the capillary column was attached to the high pressure injector used for column flushing (Supplementary Material, Appendix C). The capillary columns were chromatographically evaluated using a standard set of electroactive compounds (L-ascorbic acid, hydroquinone, resorcinol, catechol, 4-methyl catechol). L-ascorbic acid was used as the void time marker and all other components were 400 μM. A pressure injection was made at around 1000 psi for 3 s using a Haskel (Burbank, CA) DSTV-100 pneumatic amplifier pump, after which time the sample was flushed from the injector and the sample was eluted at high pressure using the DSXHF-903 pneumatic amplifier pump using a mobile phase consisting of 60/40 v/v water/acetonitrile with 0.1% TFA at ambient temperature. The DSTV-100 and DSXHF-903 pumps were connected in series to the injector to allow for the injection and elution events. The analytes were amperometrically detected at the column outlet using an 8 μm diameter carbon fiber microelectrode held at +1.1 V relative to an Ag/AgCl reference electrode [22]. Data acquisition and h–ν plot creation was performed as previously reported [14]. Efficiency data are reported for hydroquinone ($k' \sim 0.2$) by analyzing the chromatograms using the method of iterative statistical moments with $\pm 3\sigma$ integration limits using Igor Pro 6.0 software from Wavemetrics, Inc. (Lake Oswego, OR), as previously reported [23]. Reduced minimum plate height and corresponding efficiency are reported from fitting experimental data with a reduced van Deemter equation using Igor Pro 6.0. In all cases, the fits matched the data well and were therefore judged to be representative, allowing calculation of minimum reduced plate heights (h_{min}) from the fits.

3. Results/discussion

3.1. Column preparation

For the 30 μm ID columns packed at 30 mg/mL (Fig. 1A), it can be seen that the packing progress plots for the four columns overlay each other very well. When the study at 30 mg/mL in a 30 μm ID capillary was repeated, the first column stopped packing early

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