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Preparation of high-efficiency HILIC capillary columns utilizing slurry packing at 2100 bar

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ABSTRACT

Complex mixture analysis requires high-efficiency chromatography columns. Although reversed phase liquid chromatography (RPLC) is the dominant approach for such mixtures, hydrophilic interaction liquid chromatography (HILIC) is an important complement to RPLC by enabling the separation of polar compounds. Chromatography theory predicts that small particles and long columns will yield high efficiency; however, little work has been done to prepare HILIC columns longer than 25 cm packed with sub-2 μm particles. In this work, we tested the slurry packing of 75 cm long HILIC columns with 1.7 μm bridged-ethyl-hybrid amide HILIC particles at 2,100 bar (30,000 PSI). Acetonitrile, methanol, acetone, and water were tested as slurry solvents, with acetonitrile providing the best columns. Slurry concentrations of 50–200 mg/mL were assessed, and while 50–150 mg/mL provided comparable results, the 150 mg/mL columns provided the shortest packing times (9 min). Columns prepared using 150 mg/mL slurries in acetonitrile yielded a reduced minimum plate height (h_{min}) of 3.3 and an efficiency of 120,000 theoretical plates for acenaphthene, an unretained solute. Para-toluenesulfonic acid produced the lowest h_{min} of 1.9 and the highest efficiency of 210,000 theoretical plates. These results identify conditions for producing high-efficiency HILIC columns with potential applications to complex mixture analysis.

1. Introduction

High-efficiency separations are required to analyze complex mixtures, e.g., samples encountered in proteomics, lipidomics, metabolomics, and environmental studies. Although reversed phase LC (RPLC) is the dominant method in such analyses, hydrophilic interaction liquid chromatography (HILIC) is often necessary to obtain more in-depth analysis for samples containing polar analytes [1–4]. The complexity of such mixtures dictates a need for high-efficiency HILIC columns. In this work, we explore conditions for achieving high-efficiency HILIC separations based on slurry-packing 75 cm long HILIC columns.

Numerous avenues are being pursued to improve separation efficiency for LC. One approach is to use columns longer than standard for analytical scale separations (e.g., 30–400 cm length). When using longer columns, higher pressure is required, a technical hurdle that has been overcome with ultrahigh-pressure LC instruments [5–9]. Alternatively, column beds with high permeability, such as monoliths or microfabricated arrays, can be used to generate long columns [10–13]. For packed beds, smaller particles can be used to generate higher efficiency, with considerable work currently done with 1.7–2.0 μm particles and examples of smaller particles having been reported [14–19]. Long columns packed with sub-2.0 μm particles have generated high efficiencies and peak capacities, translating to improved analyte detection in complex mixtures due to reduced coelution and ionization suppression (for MS detection) despite the dilution from longer columns [20–26]. These improvements have primarily focused on RPLC columns.

Several approaches have been used to obtain high separation efficiency by HILIC. Coupling multiple short analytical HILIC columns can achieve high-efficiency separations within commercial instrument pressure regimes but remains a costly option [27]. Long monolithic capillary columns with high permeability have also been prepared [11,12,28–30], but these columns typically have low sample loading capacity and require excellent control of synthesis conditions [14,31,32]. Perhaps the highest performance by HILIC used a 4 m long monolithic column to achieve a plate height minimum (H_{min}) of 12 μm, efficiency of 300,000 theoretical plates (N), and a peak capacity of 360 in 4 h [30]. HILIC columns packed with 5 μm amide particles have achieved a H_{min} of 20 μm for a 15 cm column and a peak capacity of 130 over an 11 h gradient using a 100 cm column [33]. Several capillary scale HILIC separations have also been demonstrated for proteomics and other

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applications without reported efficiencies [34–36]. Little work has been done to develop long packed beds with sub-2 µm HILIC particles. Given the significant need for high-resolution separations of polar analytes, the availability of ultrahigh-pressure systems, and the excellent performance of long RPLC columns packed with sub-2 µm particles, we explored the potential for generating similar HILIC columns.

Suitable packing conditions are crucial for preparing high-efficiency packed bed columns [37–39]. Electroosmosis, centripetal forces, and pressure have all been utilized for slurry packing capillary columns [25,26,38–43]. Several variants of pressure-based slurry-packing are used. Long capillary columns (50–800 cm) with sub-2 µm particles have been prepared using low-pressure (100 bar) with highly concentrated slurries (500–1000 mg/mL) [44]. Similar column lengths have been prepared with ultrahigh-pressure of 2070–3450 bar and 3–200 mg/mL slurries [45]. Effects of slurry composition, sonication, and column aspect ratio on column efficiency have been explored at ultrahigh-pressures for RPLC columns [46–50]. Of these approaches, high-pressure slurry packing has been the most commonly used for long, high-efficiency RPLC columns.

Here, we report ultrahigh pressure slurry packing of efficient 75 cm long columns using 1.7 µm particles with a bridged-ethyl-hybrid (BEH) amide stationary phase, resulting in the lowest plate heights reported for long HILIC columns. The results expand the potential of HILIC for complex mixture analysis.

2. Materials and methods

2.1. Chemicals and materials

Acenaphthene (Ace), caffeine (Caf), para-toluenesulfonic acid (PTSA), nicotinamide (Nic), cytidine (Cyt), ammonium formate, formic acid, fluorescein, formamide, and the LC grade solvents acetonitrile, methanol, acetone, hexanes, and water were purchased from Sigma-Aldrich (St. Louis, MO). Kasil 1, Kasil 1624, and Kasil 2130 were acquired from PQ Corporation (Malvern, PA). A Corning Costar 96-well plate was purchased from Fisher Scientific (Pittsburg, PA). Polyimide-coated fused-silica capillary with a 360 µm outer diameter and 20 or 100 µm inner diameter was purchased from Polymicro Technologies, Inc. (Phoenix, AZ). 1.7 µm BEH amide particles were donated by Waters Corporation (Milford, MA).

2.2. Frit preparation

A summary of outlet frit preparation is illustrated in Fig. S1. Briefly, a 9:3:3:1 mixture of Kasil 1624, Kasil 1, formamide, and 7.5 mM fluorescein solution was vortexed and centrifuged in an Eppendorf MiniSpin centrifuge for 5 min at 13,400 rpm. 150 µL of the fritting solution supernatant was then transferred to one individual well of a flat-bottomed 96-well plate. Hexanes (~150 µL) was pipetted above the fritting solution and periodically added as evaporation occurred. The fritting solution and hexanes remained immiscible, leaving two separate layers. Another well was filled with 300 µL of water. A Chemyx Inc. Fusion 200 (Stafford, TX) syringe pump equipped with a 50 µL Hamilton syringe (Reno, NV; PN 80,975) filled with water was connected to a ~155 cm × 100 µm capillary through a four-port LabSmith AV202-C360 valve (Livermore, CA). The flow rate of the syringe pump was set to 250 nL/min in withdraw mode. The other end of the capillary was attached to a 3-axis computerized numerical controller (CNC) and positioned such that at its lowest z-axis position, the capillary end would be in the fritting solution. The CNC followed a pre-written script through Universal G-Code Sender software, which sequentially moved the capillary to the hexanes layer for 20 s, fritting solution for 0.9 s, hexanes layer for 20 s, and then the water well for 6 min. This protocol resulted in a ~2 mm length of frit solution being withdrawn to 20 cm from the capillary end. The capillary was removed from the system by raising the capillary end to air, stopping the flow, opening the valve to air, and slowly removing it from the LabSmith valve. The fritted capillaries were allowed to sit on a level surface at room temperature for 2–3 days before being transferred to a programmable column oven. Once placed in the oven, a 40–100 °C temperature ramp (0.5 °C/min) evaporated hexanes slowly. The fritted capillaries were left at 100 °C overnight.

Inlet frits were formed after column packing using a previously described procedure utilizing Kasil 2130 and formamide [51]. Briefly, 10 µL of Kasil 2130 and 10 µL of formamide were pipetted onto a glass microfiber filter (Reeve Angel; Clifton NJ). The capillary tip was dabbed several times on the wetted paper. The fritted capillaries were placed in a column oven at 50 °C overnight.

2.3. Column packing

Slurry packing of capillary columns by ultrahigh-pressure and the equipment required have been previously reported [8,37]. The method was modified slightly to pack 1.7 µm BEH amide particles. Briefly, the inlet of a 100 µm diameter capillary, equipped with an outlet frit, was submerged into 0.5 mL of the slurry but above the stir bar within a packing reservoir with 0.96 mL internal volume [8]. The slurry was displaced into the capillary using acetone delivered via a pneumatic amplifier pump capable of 3400 bar (Haskel; Burbank, CA) through 4100 bar compatible HiP valves (Erie, PA). The rest of the capillary was placed into an ultrasonic bath (Elma; Singen am Hohentwiel, Germany) pulsed at 80 kHz. Ultrasonication of the capillary bed has previously been reported to prevent column voids from forming with high slurry concentrations during packing [49]. The first 2 cm of the capillary were packed at ~140 bar before the pressure was quickly ramped to 2100 bar until the desired column length was achieved. The capillary was allowed to depressurize for 30 min before being attached to the isocratic system (described in more detail in the next section) and subjected to 3400 bar flow of 50:50 acetonitrile:water for 45 min. The capillary column was removed from the isocratic system after depressurizing for 1 h. Several days later, the capillary column was cut at the desired length before an inlet frit was prepared. A transparent 2 cm window for UV detection was prepared by stripping the polyimide coating at the outlet frit position with a butane lighter.

2.4. Ultrahigh-pressure isocratic separations and data analysis

Capillary columns were assessed using an isocratic ultrahigh-pressure LC system described previously [52]. Two pneumatic amplifier pumps with maximum outlet pressures of 1000 and 5200 bar were connected in series and provided pressure for injection and separation, respectively. The mobile phase consisted of 85:15 acetonitrile:water with 10 mM ammonium formate and 0.1 % v/v formic acid. The mobile phase pH was 5.85, and the viscosity was 4.8×10^{-4} Pa s. A sample mixture of 0.2 mM Ace, 1.5 mM Caf, 1 mM PTSA, 2 mM Nic, and 2 mM Cyt in the same composition as the mobile phase was utilized to assess column performance. 500 µL of sample was pushed through the HiP valves by syringe to fill the entire injection loop. The lower pressure pump, pre-set to 69 bar, was turned on for 1 s to inject a small sample plug onto the column (~ 0.5 nL). The injection loop was then opened to flush the remaining solution out of the loop and closed again before applying the pre-set separation pressure. A brief pressure drop (~3 s) was observed when the injection loop was in the open position; however, the pressure stabilized within several seconds after the higher-pressure separation pump was utilized. Operating pressures from 100 to 2100 bar were used for van Deemter curves. The outlet frit was positioned upstream of the flow cell on a Thermo Separation Products SC100 capillary ultraviolet detector to minimize post-column dispersion. Absorbance at 224 nm was reported as the voltage output of the detector. Data were collected at 1–20 Hz with a NIST digital-to-analog converter. A 10 cm × 20 µm empty capillary was attached post-column to maintain a more stable chromatographic baseline.

Peak widths and theoretical plate measurements were calculated by statistical moments through Igor Pro-software as described previously [17]. Eddy dispersion (A-term), longitudinal diffusion (B-term), and the mass transfer (C-term) were determined from a regression fit to the van Deemter equation (linear velocity versus plate height) applied by Igor Pro.

For calculating flow velocity, Ace was used as a void time marker (t_0). A previous study with the same stationary phase and similar mobile phase to that used here showed that Ace elutes ~13 % faster than acetonitrile, likely due to being partially excluded from the pores [53]. Therefore the t_0 reported here may be slightly underestimated [53]. Flow velocities used for the van Deemter graphs were not corrected for this error.

2.5. Analytical scale LC separations and reduced parameter measurements

Control separations on an analytical scale column (Waters 2.1 × 100 mm; 1.7 µm BEH amide) for comparison to capillary scale separations were performed using an Agilent 1290 Infinity II UHPLC equipped with a diode array detector and 10 µL flow-cell. Analyte absorbance was similarly monitored at 224 nm at a frequency of 5 Hz.

Diffusion coefficients (D_m) for conversion to reduced parameters were measured by peak parking experiments [54] on the analytical scale column. Particle diameter (d_p) was measured by imaging samples of particles using a JEOL JSM-7800FLV scanning electron microscope (SEM). A 2 mg/mL slurry of the particles was prepared in acetonitrile before being transferred to carbon tape on an aluminum specimen mount. The particles were allowed to dry and were subsequently sputter-coated with gold for 90 s before introduction to the SEM chamber.

3. Results and discussion

3.1. Column characterization using reduced parameters

The average particle diameter (d_p) of the stationary phase particles and the D_m for test analytes were measured to allow evaluation of columns via reduced plate height (h) and velocity (v) [55,56]. The average d_p for the BEH amide particles was 1.9 ± 0.3 µm (n = 108) by SEM analysis (Fig. S2). Similar particle size distributions (0.06–0.21 µm standard deviation for 2.7–3.9 µm particles) have been observed for fully porous particles [57], with sub-2 µm stationary phase particles often having broader size distributions [15,58]. The D_m for Ace, Caf, Nic, and Cyt were derived from peak-parking experiments (Fig. S3) [54]. D_m for PTSA was estimated as the average of the other analytes (1.24×10^{-5} $cm^2\ s^{-1}$) as coelution with Ace, Caf or Nic depending on the park time was observed when included in the mixture which prevented an accurate D_m from being calculated. This effect was presumably due to interaction with metal surfaces of the column and the negative character of PTSA with the mobile phase utilized (pH 5.85) [59].

3.2. Effect of slurry composition on kinetic performance

The choice of slurry solvent for column packing is an essential variable to consider when preparing packed columns. Acetone, water, methanol, and acetonitrile were assessed as potential slurry solvents. Reduced van Deemter curves for 75 cm long capillary HILIC columns packed with 150 mg/mL concentration slurries are shown in Fig. 1. Good repeatability was achieved for two column replicates prepared with each solvent. The choice of slurry solvent had a profound effect on column performance. The best-performing columns were achieved with acetonitrile as the slurry solvent, followed by methanol, water, and acetone. These results contrast those for packing RPLC porous particles, where acetone slurries produced the most efficient columns [49,52].

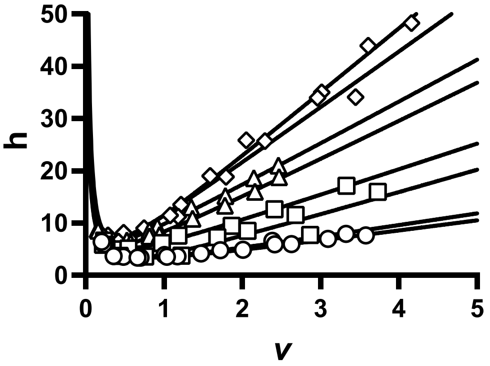

Fig. 1. Effect of slurry solvent on reduced van Deemter curve for unretained Ace. 75 cm long × 100 µm ID capillary columns were prepared with acetone (diamonds), water (triangles), methanol (squares), or acetonitrile (circles) slurry solvent. van Deemter equation fit lines are shown for all columnse. Particle concentrations were 150 mg/mL of slurry. The mobile phase was 85:15 acetonitrile:water with 10 mM ammonium formate and 0.1 % v/v formic acid.

To better understand these effects, slurries with different solvents were examined for aggregation using optical microscopy as higher aggregation of particles has produced improved column quality with capillary column dimensions [37–39,43,46]. This observation is believed to be due to aggregates leading to less particle size segregation and particle rearrangement during packing relative to dispersed slurries [38,49,60]. As shown in Fig. S4, optical microscopy images of a 0.5 mg/mL slurry taken near the evaporation edge of the solvent showed that acetonitrile was the most aggregating solvent while acetone was the most dispersive. The result of more aggregating solvents promoting good performance for ultra-high pressure slurry packing of capillary columns is in agreement with previous studies focused on RPLC columns [46,47,49]. This work shows that observation also extends to HILIC.

Although the good performance of acetonitrile can be attributed to aggregation, aggregation was not the only solvent property in determining column quality. For example, methanol slurries produced better columns than water even though water produced more particle aggregation. The relatively poor performance of water relative to methanol may be due to other solvent properties affecting column performance. As discussed before, solvent properties such as surface tension, viscosity, and density (summarized in Table S1) can also play a role in observed column performance [39]. Some observations are helpful in possibly understanding the results with water. The packing time was over four times longer with water than the other solvents. Water also produced columns with slightly higher retention factors than other solvents e.g., nicotinamide had $k = 0.73$ for a water packed column but between 0.61 and 0.62 for the other solvents. This higher k is suggestive of higher packing density [61]. The slow packing may relate to both the higher viscosity and the development of a more dense packed bed since packing was done at a constant pressure. A more detailed study on effects of solvents on packed bed structure for HILIC would be required to elucidate these effects, especially since HILIC may not follow the same trends as RPLC [37]. Such studies may be fruitful for further improving performance of packed capillary HILIC columns.

The solvents screened were selected because they are commonly utilized in HILIC mobile phases. Other water-miscible polar organic solvents, such as tetrahydrofuran or mixtures of solvents, may yield more efficient HILIC capillary columns but were not explored. Acetonitrile as a slurry solvent is further advantageous compared to the other solvents explored, as acetonitrile-rich mobile phases are recommended for column equilibration and storage for the BEH amide particles [62].

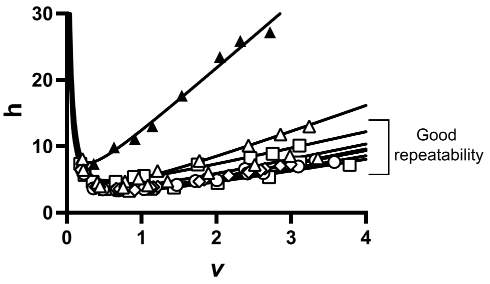

Fig. 2. Reduced van Deemter curves as a function of slurry concentration. 75 cm × 100 μm capillary columns were prepared at a concentration of 50 (triangles), 100 (squares), 150 (black circles), or 200 mg/mL (diamonds) in acetonitrile. Two replicates are shown except for the 200 mg/mL slurries, where three replicates are plotted. van Deemter equation fit lines are shown for all columns. The first and second column replicate are shown with open symbols while the third 200 mg/mL column replicate is shown with closed symbols. The analyte was Ace. The mobile phase was 85:15 acetonitrile:water with 10 mM ammonium formate and 0.1 % v/v formic acid. Good repeatability was observed for 50–150 mg/mL slurry concentrations.

Slurry concentration is another critical factor in column performance. The best slurry concentration typically exists between two extremes. At too low of slurry concentrations, smaller particles congregate to the walls of the column, leading to radial heterogeneities and reduced performance [47,60]. At too high of slurry concentrations, voids in the bed can be formed, especially with ~1 μm particles that exhibit high cohesive and frictional forces [16,47]. Concentrations ranging from 50 to 200 mg/mL were assessed for acetonitrile-based slurries (Fig. 2). Similar separation efficiencies were obtained for 50, 100, and 150 mg/mL slurries. At a concentration of 200 mg/mL, poor repeatability was observed. Higher slurry concentration promoted faster packing. Packing times for a 75 cm long capillary were 62, 15, 12, and 9 min for 50, 100, 150, and 200 mg/mL slurries, respectively. Based on these combined results, packing HILIC capillary columns with a 150 mg/mL acetonitrile slurry was chosen as column preparation is relatively quick with good repeatability and separation efficiency.

Reduced van Deemter coefficients for the best performing HILIC columns ($h_{min} = 3.3$; and maximal theoretical plates or $N_{max} = 120{,}000$ for Ace with $k = 0$) were: $a = 0.39$, $b = 1.17$, and $c = 1.85$. For comparison to reversed phase LC, it has been reported that 60 cm × 75 μm capillaries packed with 1.7 μm BEH C18 particles yielded reduced coefficients of $a = 0.41$, $b = 1.52$, and $c = 0.15$ for a test analyte with $k' = 0.2$ [22]. (These columns were packed under similar conditions but used 200 mg/mL slurries with acetone as the solvent, the best conditions for RP columns). The lower b-term and higher c-terms found with HILIC relative to RPLC columns has been observed and studied before [33,63–66]. It has been suggested that the lower b-term may relate to slower surface intraparticle diffusion in HILIC than RPLC. The higher c-terms may relate to slower transverse diffusion in HILIC which prevents evening out of concentration gradients induced across the column [63–65]; however, this effect would be expected to be small in a narrow bore column such as used here. A role for intraparticle structure in increasing the observed c-term has been identified as well with HLIC columns resulting in worse kinetic properties than RPLC columns [66]. As discussed previously, the practical effect is that although comparable h_{min} is achievable and therefore maximal plates by HILIC and RPLC, faster analyses that use higher flow rates will be higher efficiency by RPLC than by HILIC [65].

3.3. Analyte and commercial column comparisons

Sample chromatograms from testing these columns are shown in Fig. 3, and analyte-specific van Deemter curves are shown in Fig. 4. Most compounds performed similarly, although the unretained test analyte, Ace, had slightly worse performance than the other test compounds. The higher h values for the least retained Ace suggest that extra column effects may contribute to h despite efforts to minimize dead volumes. PTSA was notable, having lower h values throughout the velocity range and lowest increases with flow rate, resulting in a relatively flat van Deemter curve. PTSA had a h_{min} of 1.9, corresponding to 210,000 theoretical plates. The differences in performance among the retained compounds may be due to differences in molecular properties, as previously observed for HILIC [27,67]. The high performance of PTSA is possibly due to it being completely ionized in solution as a strong acid, as these previous studies have indicated that ionized species perform well in HILIC. At the same time, PTSA was more variable. It is believed that anionic interactions with metal surfaces of the UHPLC instrument contributed to the poor repeatability of PTSA [59].

Next, we compared the kinetic performance of the long capillary column to a commercially available 2.1 inner diameter × 100 mm long column with the same 1.7 μm BEH amide particles for Caf and Nic (Fig. 5). Similar plate heights were observed over the linear velocities assessed, suggesting that the quality of the packed bed was similar for both columns. However, the longer capillary column yields up to a ~7.5-fold improvement in theoretical plates at a given reduced velocity but at the cost of longer retention time. For comparable retention times, the performance is also better for the longer column. For example, the retention time of Caf on a 75 cm long column at 2100 bar is similar to that achieved by a commercial column operated at 40 bar; however, the peak width of Caf is 42 % less for the long capillary column ($N = 46{,}000$) than the commercial column ($N = 23{,}000$) (Fig. 5C).

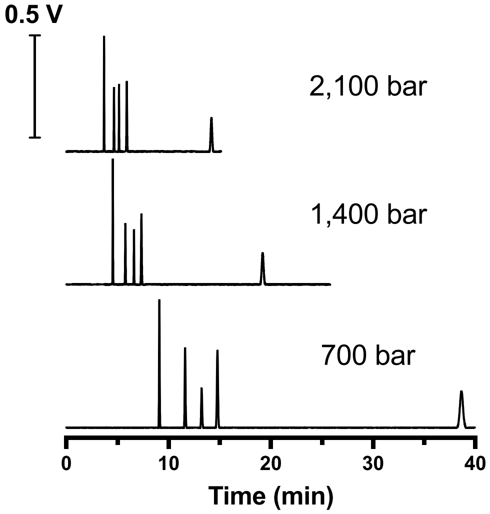

Fig. 3. Chromatograms of the HILIC test mixture using UV Absorbance detection were obtained on a 75 cm × 100 μm capillary packed with 1.7 μm BEH amide particles at operating pressures indicated. Absorbance intensity is expressed as a function of voltage (V). The elution order is Ace, Caf, PTSA, Nic, and Cyt. The number of theoretical plates for Ace were 105,000, 71,400, and 56,800 at operating pressures of 700 bar, 1400 bar, and 2100 bar, respectively. The mobile phase was 85:15 acetonitrile:water with 10 mM ammonium formate and 0.1 % v/v formic acid.

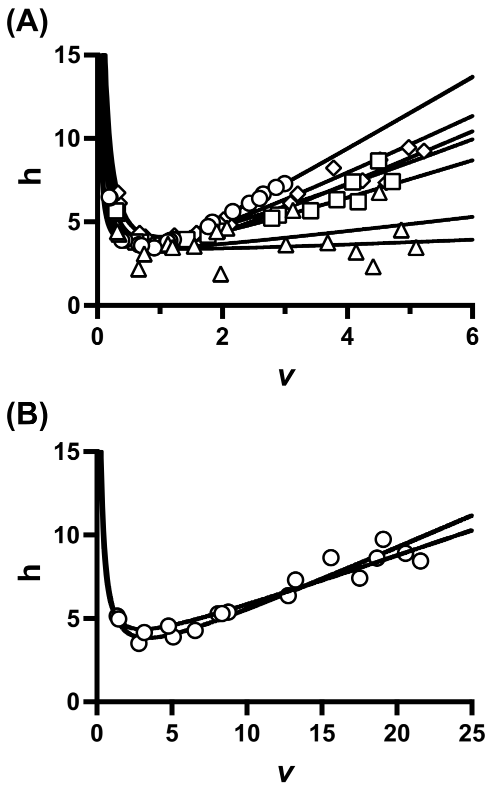

Fig. 4. Reduced van Deemter curves for HILIC test mixture compounds (A) Ace (circles), Caf (squares), PTSA (diamonds), and Nic (triangles). (B) Reduced van Deemter curves for Cyt. Two column replicates are shown for each analyte and solid van Deemter equation fit lines are provided. The mobile phase was 85:15 acetonitrile:water with 10 mM ammonium formate and 0.1 % v/v formic acid.

3.4. Ultrahigh-pressure HILIC separations

In this study, Packed capillary columns were operated at pressures as high as 2100 bar (30,000 PSI) to obtain van Deemter curves, assess the particle durability, and examine feasibility of fast HILIC separations. During these experiments, we observed that the retention factor decreased with increasing operating pressure, with the effect most notable for the most retained analyte (Cyt, Fig. 6). This trend is in agreement with previous observations of HILIC separations and the opposite of what has been observed for RPLC [8,68,69]. Previous studies suggested that retention may decrease with pressure due to increases in temperature, associated with higher flow rate due to frictional heating, as well as direct effects of pressure on retention mechanisms [68,70–72]. The high surface area to volume of ratio likely limits temperature related effects in the capillary columns used here. The increase in retention for sugars with pressure on amide phases has been attributed to a gain in solvation as solutes partition from acetonitrile rich mobile phases into an adhered water stationary phase [69]. This effect is a likely explanation for the strong effect on ribose-containing cytidine.

4. Conclusions

We investigated packing conditions for developing efficient 75 cm long HILIC capillary columns packed at ultrahigh-pressure with 1.7 μm BEH amide particles. HILIC capillary columns prepared with acetonitrile-based slurries produced a $H_{min} = 6.2$ μm, $h_{min} = 3.3$, and 120,000 theoretical plates (160,000 $N\ m^{-1}$) for an unretained compound and better performance for retained species. These are the lowest plate heights reported for long HILIC columns and, as a result, present good efficiency per time compared to previous studies of long HILIC columns [30,33]. These results demonstrate the feasibility of packing long HILIC columns with sub-2 μm particles to improve separation performance compared to commercially available columns. While it may be possible to pack standard bore sizes of 1–4 mm inner diameter at such lengths, using capillaries reduces the amount of packing material needed, reduces mobile phase consumption, facilitates interface to mass spectrometry, and enables smaller sample analysis.

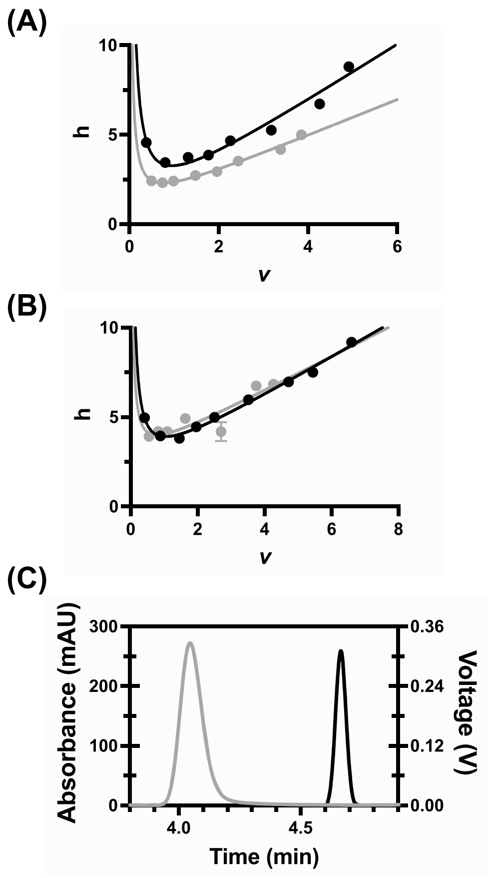

Fig. 5. Reduced van Deemter curves were obtained with a 2.1 × 100 mm commercial column (gray) and 75 cm × 100 μm home-packed capillary column (black) for (A) Caf and (B) Nic. Error bars signify one standard deviation with triplicate analysis. van Deemter Lines of best fit are shown. (C) Caf had a similar retention time by commercial chromatography at 40 bar ($N = 23{,}000 \pm 680$; left y-axis) and at 2100 bar separation ($N = 46{,}000$; right y-axis) with a 75 cm × 100 μm column. The mobile phase was 85:15 acetonitrile:water with 10 mM ammonium formate and 0.1 % v/v formic acid.

Interestingly, the h_{min} was achieved at 390 bar, a relatively low pressure for such a long column. This low pressure is attributed to the low viscosity of the mobile phase (4.8×10^{-4} Pa s). Therefore, columns of this length are compatible with the pressure limits of commercially available LC pumping systems for a reasonable flow rate range. It also suggests that, given the potential to operate up to 3100 bar [20,24], even longer columns or smaller particles may be feasible. Such HILIC columns may benefit the analysis of complex samples found in omics, environmental, and forensic applications.

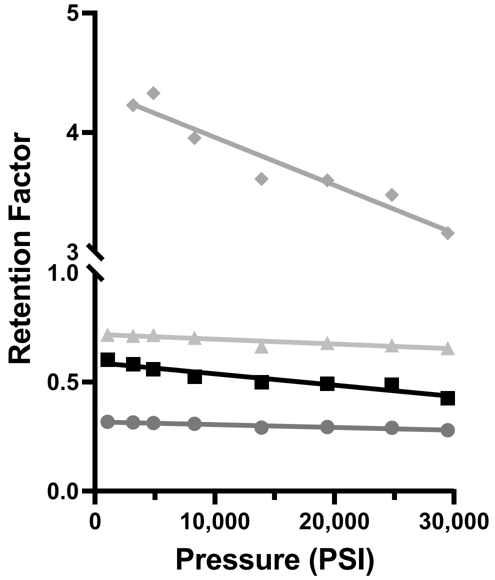

Fig. 6. Retention factor versus operating pressure for (circles) caffeine, (squares) PTSA, (triangles) nicotinamide, and (diamonds) cytidine. A negative linear regression is observed for all analytes. The mobile phase was 85:15 acetonitrile:water with 10 mM ammonium formate and 0.1 % v/v formic acid.

Supplemental information

A schematic of the fritting procedure utilized during column fabrication, particle sizing, peak parking, and sedimentation velocity results are included. Several physical properties of the slurry solvents screened are also included for reference.

CRediT authorship contribution statement

Brady G. Anderson: Investigation, Writing – original draft, Writing – review & editing. **Tate A. Hancock:** Formal analysis, Investigation, Writing – review & editing. **Robert T. Kennedy:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Robert T. Kennedy reports financial support was provided by the National Science Foundation. Robert T. Kennedy reports financial support was provided by the National Institutes of Health. Robert T. Kennedy reports equipment, drugs, or supplies was provided by Waters Corporation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2024.464856.

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