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Characterizing pressure issues due to turbulent flow in tubing, in ultra-fast chiral supercritical fluid chromatography at up to 580 bar

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A B S T R A C T

It has been widely suggested that the outlet pressure be changed to maintain constant density ("isopycnic" conditions) when comparing the kinetic performance of different columns in supercritical fluid chromatography (SFC). However, at high flow rates, flow in the tubing is turbulent, causing large extra-column pressure drops that limit options for changing outlet pressure. Some of these pressure drops occur before and some after the column, obscuring the actual column inlet and outlet pressures.

In this work, a 4.6 × 100 mm, 1.8 μm R,R-Whelk-O1 column was used with low dispersion LD (120 μm) plumbing to generate sub-1 min chiral separations. However, the optimum, or near optimum, flow rate was 5 mL-min^{-1}, producing a system pressure of 580 bar (with 40% methanol, outlet pressure 120 bar). Both the flow rate and pump pressure required were near the limits of the instrument, and significantly exceeded the capability of many other SFC's. Extra-column pressure drops (ΔP_{ec}) were as high as 200 bar, caused mostly by turbulent flow in the tubing. The ΔP_{ec} increased by more than the square of the flow rate.

Reynolds Numbers (Re) were calculated for tubing as a function of flow rate between 100 and 400 bar and 5–20% methanol in CO_2, and 40°–60 °C. This represents the most extensive analysis of turbulence in tubing in the SFC literature. Flow in 120 μm ID tubing was calculated to be laminar below 1.0 mL-min^{-1}, mostly transitional up to 2.5 mL-min^{-1} and virtually always turbulent at 3 mL-min^{-1} and higher. Flow in 170 μm tubing is turbulent at lower flows but generates half the ΔP_{ec} due to the lower mobile phase linear velocity.

The results suggest that, while sub-minute chromatograms are easily generated, 4.6 mm columns are not very user friendly for use with sub-2 μm packings. The high flow rates required just to reach optimum result in high ΔP_{ec} generated by the tubing, causing uncertainty in the true column inlet, outlet, and average column pressure/density. When comparing kinetic performance of columns with different dimensions, the pressure drops in the tubing must be considered.

1. Introduction

The use of sub-2 μm particles in supercritical fluid chromatography (SFC) is still largely in its infancy. This is primarily due to the fact that the standard plumbing in current commercial SFC instruments was not designed to produce the full theoretical efficiency with such particles. Most reports with sub-2 μm achiral columns, using such SFC's produced reduced plate heights (h_r) ≈3 or higher [1–5]. Extra-column dispersion is on the order of 90 μL^2 [4].

In ultra high performance liquid chromatography (UHPLC) h_r near 2 are relatively common with sub-2 μm particles. In order for SFC to match this performance, modifications to the standard plumbing of commercial SFC's is required, in order to decrease extra-column dispersion by at least an order of magnitude. This was accomplished in a recent report [6] demonstrating full "theoretical" performance ($h_r < 2$, and 95% of theoretical at $k = 2$) with bare silica columns when the standard plumbing (STD) was replaced with shorter lengths of smaller ID tubing, and a smaller volume flow cell, creating a low dispersion plumbing (LD) configuration. With 3 × 100 mm columns, packed with 1.8 μm particles, the pump pressure needed to operate near the optimum flow rate of 1.7–2 mL-min^{-1} was 325 bar (outlet pressure 150 bar). Without the column, ΔP_{ec} was on the order of 20 bar (at 2 mL-min^{-1}). However, when generating vanDeemter-like plots of flow rate vs. h_r, pressure increased non-linearly with increased flow. The ΔP across the system without a column, at 5 mL-min^{-1}, was found to be on the order of 180 bar. Thus, the extra-column ΔP increased ≈9 times for

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an increase in flow of ≈2.5 times. The maximum pump pressure exceeded 520 bar, but since the pump was capable of 600 bar, the tubing ΔP seemed surprising, but irrelevant. At the time it was not recognized what the full implications of the non-linearity or the very large extra-column ΔP at high flows meant.

With laminar flow, typical in HPLC, the ΔP in the tubing is linearly proportional to the flow rate (Poiuselle Eq.). With turbulent flow, the ΔP in the tubing is proportional to at least the square of the flow rate [7]. The very large increase in the extra-column ΔP with the higher flows, strongly suggests that the flow in the tubing was increasingly turbulent at higher flows.

There is limited characterization in the literature of the degree of mobile phase turbulent flow in the connector tubing in SFC [7,8]. Much of what exists was generated with pure CO_2. The addition of modifier increases the mobile phase viscosity, and decreases the solutes diffusion coefficients, making SFC with modified mobile phases somewhat more like HPLC with normal fluids.

A recent report [9] calculated kinematic viscosity of methanol modified CO_2 over a range of common temperatures and pressures used in SFC (40–60 °C, 100–400 bar, 5–20% methanol). The main purpose there was to calculate pressure and density gradients along the length of the column. Some of this data was alternately used [8] to calculate Re numbers in various sized tubing under the limited case of 20% methanol, at 2.4 mL-min^{-1} and room temperature. In 127 μm ID tubing, the Re was 3015, indicating the flow was transitional. One of the goals of this work was to further characterize the nature of the flow under a wider range of conditions typically used in SFC. The kinematic viscosities from [9] were used to calculate expanded Re numbers in both the LD and Std. stainless steel tubing, as a function of flow, temperature and% modifier under typical SFC conditions. Although important, the surface roughness of the tubing was not considered.

In HPLC, pressure has little effect on retention, k, density, ρ, and solute diffusion coefficients, D_m. In SFC, k, ρ, and D_m all change at least moderately with pressure, particularly when using low polarity solutes and pure or only slightly modified (a few% modifier) CO_2 as the mobile phase. Many authors are concerned about the effect of changes in pressure on k, ρ, D_m, in method transfer, and generation of vanDeemter curves [1,9–18], but appear to be unaware of, or unconcerned by, large ΔP_{ec} caused by the tubing. Large ΔP_{ec} in connector tubing obscures the actual pressure drop across the column. In particular, the extra-column pressure drop after the column distorts both the true column outlet pressure and the average pressure/density in the column. Such ΔP_{ec} also limits the length of columns that can be used with a specific pumping system. On the other hand, many of these concerns were shown to result in only minor effects [2].

Only a few reports of the use of sub-2 μm particles for chiral SFC separations have appeared, with the emphasis on very fast chromatography with very short columns [8,17–24]. None of these columns have been commercially available but some should soon be. Columns as short as 10 or 20 mm have been used, typically generating low total efficiencies. The generation of sub-1 min chromatograms is, apparently, the current goal for supporting very high throughput screening and reaction monitoring. Welch [20] has generated a chiral separation in only 5 s with SFC (using 3 μm particles). Such very fast separations require appropriate selectivity so that the relatively low efficiency of such short columns, at high flows, is adequate to generate the resolution required. Surprisingly, many very fast separations have been performed [19,22–24] using 4.6 mm ID columns packed with sub-2 μm columns, which have been shown, in at least 1 instance, to require optimum flow rates of near 5 mL-min^{-1} or higher [19].

Longer columns, such as 5 and 10 cm, can potentially yield much higher efficiencies, while maintaining high speed, compared to 10–20 mm columns. In fact, a chiral 4.6 × 50 mm column packed with 1.8 μm totally porous particles was also shown [19] to produce full theoretical efficiency ($h_r < 2$), with the same low dispersion plumbing used in [6]. Isocratic efficiencies of over 14,000 plates were generated ($h_r < 2$) for both enantiomers of t-stilbene oxide. This is the highest chiral efficiency reported in the SFC literature. In addition, one chiral separation was performed in ≈ 10 s. The standard 170 μm tubing was shown to degrade efficiency by ≈20%. The optimum flow rate was found to be ≈3.5 mL-min^{-1} at 40% methanol, increasing to >5 mL-min^{-1} at 10% methanol and below. At higher methanol concentrations (40–45%), the column head pressure exceeded 500 bar (at 5 mL-min^{-1}).

In the current study, an experimentally packed 4.6 × 100 mm, 1.8 μm R,R-Whelk-O1 column was used to generate a number of sub–1 min chromatograms by SFC. Pump pressure was expected to exceed 500 bar. The source of this large ΔP was evaluated. Some effort was made to see if extra-column ΔP could be minimized.

2. Experimental

2.1. Instrumentation

A Model 4301A SFC was controlled by an OpenLabs ChemStation (Model C.01.03) all manufactured by Agilent Technologies, Waldbronn Germany. An SFC conversion module pre-compressed the CO_2 to just below the column inlet pressure. The “A” side of a binary HPLC pump, with its compressibility set to zero, accurately metered the CO_2 flow. Mechanically, the metering pump was an unmodified HPLC pump, except for check valves and pump seals. The “B” side pumped modifier exactly as in HPLC. This system is capable of up to 5 mL/min at up to 600 bar, simultaneously. However, the SFC conversion module only increases the pressure to ≈ 391 bar. At higher column head pressures (up to 600 bar), there is a gap between the booster pressure and the actual column head pressure. Since the compressibility compensation of the metering pump was set to zero, any pressure above 391 bar resulted in a less than perfect compressibility, creating pump pressure noise of up to ≈3 bar. This pressure fluctuation is largely dampened by the pneumatic resistance of the system and the capacitance of the column, creating a mechanical RC filter. Perhaps surprisingly, UV noise was only ≈ 0.2-0.3mAU at 80 Hz with pump pressure at 580 bar. Pressure fluctuations at the BPR were modest, <±0.2 bar.

A large volume degasser was used to degas the modifier. The pumps are connected to the injection valve with short lengths of 254 μm tubing to minimize extraneous ΔP. The autosampler loop was a 10 cm long piece of 120 μm tubing, with an internal volume of ≈1.25 μL. After injection, the injection loop remains in the flow path and contributed noticeably to the system ΔP.

In order to understand the effects of extra-column ΔP’s, on k, ρ and D_m, the plumbing used must be carefully and fully described. Two different basic plumbing configurations were employed, the standard (STD) and a low dispersion (LD) version. The tubing used in each configuration is listed in Table 1. A thermal control compartment (TCC) was used to control the mobile phase temperature entering the column using a pre-column heat exchanger (HX)(either std. or “mini”). The TCC is a stagnant air device. The column was isolated from contact with the walls using plastic clips, minimizing heat exchange between the column and the heat exchanger except through the incoming fluid. Since stagnant air is a poor heat exchanger the head of the column slowly equilibrated to the heat exchanger temperature. A post-column HX (either std. or “mini”), also in the TCC, matched the mobile phase temperature to the detector flow cell temperature [25]. A multi-meter with a thermocouple input was used to measure the temperature of the UV/Vis detector flow-cell to the nearest degree C. The temperature