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Chromatographic performance of microfluidic liquid chromatography devices: Experimental evaluation of straight versus serpentine packed channels☆

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ABSTRACT

We prepared a series of planar titanium microfluidic (μLC) columns, each 100 mm long, with 0.15, 0.3 and 0.5 mm i.d.'s. The microfluidic columns were packed with 1.8 μm C18 sorbent and tested under isocratic and gradient conditions. The efficiency and peak capacity of these devices were monitored using a micro LC instrument with minimal extra column dispersion. Columns with serpentine channels were shown to perform worse than those with straight channels. The loss of efficiency and peak capacity was more prominent for wider i.d. columns, presumably due to on-column band broadening imparted by the so-called "race-track" effect. The loss of chromatographic performance was partially mitigated by tapering the turns (reduction in i.d. through the curved region). While good performance was obtained for 0.15 mm i.d. devices even without turn tapering, the performance of 0.3 mm i.d. columns could be brought on par with capillary LC devices by tapering down to 2/3 of the nominal channel width in the turn regions. The loss of performance was not fully compensated for in 0.5 mm devices even when tapering was employed; 30% loss in efficiency and 10% loss in peak capacity was observed. The experimental data for various devices were compared using the expected theoretical relationship between peak capacity P_c and efficiency N; $(P_c - 1) = N^{0.5} \times \text{const}$. While straight μLC columns showed the expected behavior, the devices with serpentine channels did not adhere to the plot. The results suggest that the loss of efficiency due to the turns is more pronounced than the corresponding loss of peak capacity.

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1. Introduction

Microfluidic (μLC) liquid chromatography is presently a topic of significant research interest, particularly in fields where high-sensitivity liquid chromatography mass spectrometry (LC–MS) is employed, such as proteomics and metabolomics [1–7]. While the last two decades of research have largely focused on 75 μm i.d. nano LC columns, recent enhancements in MS instrument sensitivity permits the use of more robust capillary and microbore LC columns (0.15–1 mm i.d.) with acceptable separation performance and MS detection limits suitable for many such applications [8–12].

When μLC is performed correctly, it offers several advantages over conventional scale LC experiments. It provides enhanced detection sensitivity (assuming that the amount of sample injected on column is not proportionally reduced), lower consumption of mobile phases, and a reduction in the negative consequences associated with the frictional heat that is generated on column during chromatography [13–16]. On the other hand, there are several challenges encountered in μLC. First, due to the small peak volumes generated by a high efficiency μLC separation, extra-column contributions to peak broadening have to be carefully managed to maintain acceptable separation performance [17,18]. Second, even small sample volumes (a few microliters) may exceed the volume of a μLC column, creating injection related peak broadening [19]. The peak broadening problem is magnified in cases when the sample solvent interferes with retention (e.g. acting as strong eluent); in extreme cases peak splitting or sample breakthrough may be observed [19,20]. Last but not least, the μLC columns that

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are manufactured by laser ablation, mechanical micromachining or chemical through etching have channels with rectangular or trapezoidal shape. The impact of sharp corners on the performance of μLC devices has not been thoroughly studied yet [21–23].

μLC devices with separation channel lengths within excess of 5 cm often employ turns to fit in small planar footprints, as well as to maintain consistent points of attachment (inlet, outlet, heating, etc.) across different length devices [24]. These turns are known to be a source of peak dispersion, owing to the so-called race-track effect [25,26]: the zone at the inner wall of channel passes through the column via a shorter path than at the outer wall. This phenomena is more pronounced for wide i.d. channel devices and those employing sharp turn radii [24,27,28].

The negative impact of channel turns on peak dispersion in electrophoresis and μLC can be partially mitigated by tapering the channel width (decreasing the channel width) at the curved channel section [24,27,28]. In CE, the tapered turn can be very narrow, allowing for sharp turns, since the separation medium is a gel. In LC, the need to efficiently pack the column with particles in a slurry puts restrictions on the sharpness of the tapered turns. In a previous study, we experimentally confirmed that turn tapering (turns 2/3 the width of straight sections) is an efficient strategy for 0.3 mm i.d. μLC devices [17].

The goal of the current study is to experimentally investigate the impact of turns in μLC channels on chromatographic performance for a set of μLC devices ranging from 0.15 to 0.5 mm i.d. We packed the straight and serpentine channel devices with 1.8 um C18 particles and compared their performance to conventional 2.1 mm i.d. UPLC columns. The aim of this study was to answer several basic questions: Can we adequately pack straight and serpentine μLC devices? Does the performance of μLC devices match the performance of conventional UPLC columns? How much performance is lost due to race-track effect? Can turn tapering effectively mitigate the race-track effect for 0.3 and 0.5 mm i.d. devices?

2. Experimental

2.1. Materials and reagents

Analytes used in this study, uracil (U), acetophenone (AP), propiophenone (PP), butyrophenone (BP), valerophenone (VP), and hexanophenone (HP) were obtained from Sigma (St. Louis, MO, USA). HPLC grade acetonitrile (MeCN) was purchased from Fisher Scientific (Fair Lawn, NJ, USA). A Milli-Q water purification system (Millipore, Bedford, MA, USA) was used for preparation of HPLC mobile phases.

2.2. LC instrumentation

Chromatographic experiments on 2.1 mm i.d. columns were carried out using an I-Class ACQUITY UPLC system (Waters Corporation, Milford, MA, USA) consisting of a binary UPLC Pump (ACQUITY BSM), a column heater module employing an active preheater (ACQUITY CM-A), and a tunable UV detector equipped with a 250 nL detector cell (ACQUITY TUV). Samples were injected using a 10 nL Cheminert UHPLC internal sample injector (VICI, Houston, TX, USA).

Capillary LC and μLC columns were tested using the LC system described previously [17]. Briefly, the system consisted of a low flow binary UPLC pump (nanoACQUITY BSM), a modified ACQUITY TUV detector equipped with a 10 nL detector cell and a 10 nL Cheminert UHPLC internal sample injector (VICI, Houston, TX, USA). All fluidic connections were made with 365 μm o.d. fused silica capillaries. 0.15 mm i.d. columns were tested using a 15 μm × 25 cm inlet capillary and a 20 μm × 20 cm outlet capillary. The measured extra column system dispersion was approximately 30 nL^2 at a flow rate of 1.5 μL/min. 0.3 and 0.5 mm i.d. devices were tested using a 25 μm × 50 cm inlet capillary and a 25 μm × 40 cm outlet capillary. The extra column system dispersion of the second system was 300 nL^2 at 6 μL/min. Capillaries were face polished with a 9 μm FiberMet Abrasive disc (Buehler, Norwood, MA, USA) to ensure minimal dispersion in zero dead volume capillary-to-column connections. These connectors and fittings, designed to accommodate 380 μm o.d. or 680 μm o.d. tubing, were machined in-house from PEEK and stainless steel.

2.3. Column and μLC devices description, packing, and testing

Commercial 2.1 × 100 mm (i.d. × L) ACQUITY HSS T3 columns were obtained from Waters Corporation (Milford, MA, USA).

Research grade μLC columns were packed in our lab with ACQUITY HSS T3 1.8 μm sorbent. Briefly, 0.15–0.5 mm i.d. devices were connected to a packing bomb via 100 μm i.d. fused silica capillaries. The connections were accomplished using high pressure compatible, radially crimped stainless steel & PEEK sleeve assemblies. Column outlets were fitted by inserting a small amount of Whatman GF/F glass microfibers (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) at the outlet, which in turn was held in place by a 25 μm i.d. × 365 μm o.d. fused silica capillary secured in the fitting assembly. Chromatographic sorbent slurry was prepared at a concentration of 30 mg/mL in acetone, sonicated for 1 min, and a 1.8 mL volume was placed into the packing bomb. The packing was initiated at 35 MPa (acetone was used as the “push” solvent), ramping to 170 MPa in 30 s where it was held until packing was completed and an excess of packing material was observed overfilling the connecting inlet capillaries. Packing of 100 mm long columns/channels typically required 2–4 min of packing time.

In this work we commonly refer to 150, 300, and 500 μm i.d. μLC devices. However, the cross-sectional area of machined channels is not circular as it is in the case of fused silica capillaries. The μLC channels machined in titanium had dimensions of 152 × 119, 350 × 200, 508 × 381 μm (width × height), yielding cross-sectional areas equivalent to circular channels that are 150, 300, and 500 μm in diameter respectively. This approach enables us to readily scale comparison experiments while simplifying the machining process of our μLC devices.

Fig. 1 shows the geometry of μLC columns studied in this work. Fig. 1A and B schematically illustrate the μLC device manufacturing process; a rectangular groove is machined in the bottom titanium plate, which is subsequently aligned with a top plate containing a previously drilled hole (Fig. 1A). Pressure and heat are applied to bond the two plates together (Fig. 1B). The resultant device is a solid block of titanium containing a chromatographic channel down the middle and a hole (perpendicular to the main channel) drilled through to provide for fluidic access into the main chromatographic channel. We have adopted a term “via” for this type of geometry from printed circuit board industry and will use it throughout the remainder of this manuscript. The function of via is to enable a robust high pressure connection, which is more easy to accomplish on a flat surface than at the thin edge of μLC device. The fluidic connections for LC experiments were accomplished using fittings machined in house from black PEEK (see Fig. 1D and E). Fig. 1E show a device where both the inlet and outlet fluidic connections are accomplished by vias. Alternatively, the channel can run through to the edge of the device (staying within one layer) where a lower pressure outlet connection can be accomplished through yet another custom fitting design. In this case, a PEEK gasket is compressed by a stainless steel housing against the titanium μLC device, forming a fluidic seal to both the device face, as well as to a fused silica capillary inserted into the housing (Fig. 1D).

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