A NOVEL CHROMATOGRAPHY FORMAT FOR HIGH EFFICIENCY SEPARATIONS

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ABSTRACT

A non-porous C8 coated silicon pillar channel design is presented that allows the achievement of a high pressure and high efficiency operation for HPLC separations. Performing on-chip injection and detection and hence omitting the related dispersive sources, more than one million theoretical plates were obtained in non-retained conditions. The performance potential was also demonstrated by connecting the column to a commercial capillary HPLC system and injecting and detecting (UV-Vis) sample plugs off-chip.

KEYWORDS: pillar array, capillary chromatography, turn, high-pressure connection

INTRODUCTION

In the last few years, pillar arrays were introduced as a powerful alternative for classical packed bed columns and monoliths. While the chip format's 'particle' order induces a decrease of the plate height by a factor of 2, also the flow resistance can be drastically reduced compared to what is observed in densely packed channels [1].

Our group has recently invested a lot of efforts to reproducibly obtain performances that are in line with theoretical expectations using the relatively accessible mid-UV lithography equipment. The sidewall design and the pillar verticality appear to be, for a specific pillar bed design, crucial elements to achieve the desired efficiencies; this poses large challenges with respect to fabrication.

To exploit the performance of a column optimally, a column should have a length such that the achievable linear velocity is close to the optimum of a van Deemter curve. For the currently used design with a maximum operation pressure of 350 bar this corresponds to a channel of a few m.

EXPERIMENTAL

A well validated pillar array design containing 5 µm diameter pillars was implemented in channel tracks (Fig. 1). To achieve long channel lengths, turns are indispensible. To evenly distribute the sample at every transition between a turn channel and the much wider pillar channel (to avoid racetrack effects), distributors features [2] were implemented (Fig. 2). By coupling channel segments of a few cm in parallel micro pillar array channels, pillar arrays of up to 3 m long out of a 10 cm diameter silicon wafer were constructed (Fig. 1b). To allow for relevant pressures, dedicated channels (120 µm wide) were etched to insert 108 µm diameter capillaries into (Fig. 3), the interface was sealed by epoxy glue. Channels were coated with octyldimethylchlorosilane to perform reversed phase separations.

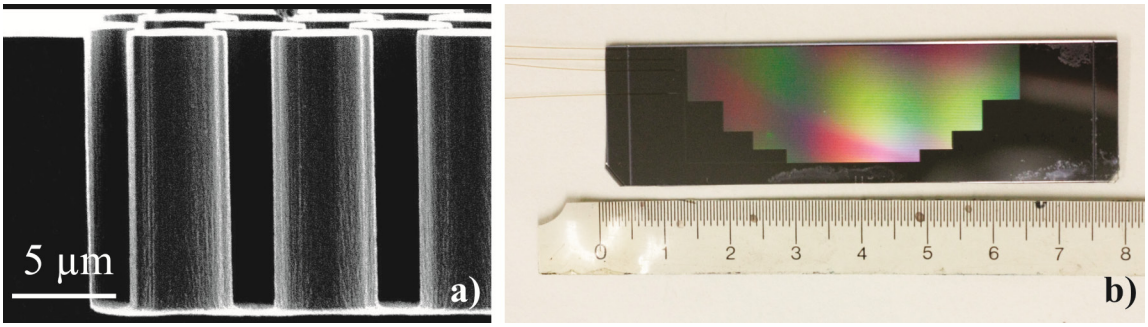

Figure 1. ***a)*** *SEM image of cross section of the pillar channel at the sidewall region containing embedded pillars. The inter pillar distance, the pillar diameter and the depth are 2.5 µm, 5 µm and 14.6 µm, respectively.* ***b)*** *Optical image of a 3 m pillar channel (channel width 150 µm, ruler in cm), including the Borofloat cover lid. In the top left corner the connection capillaries are depicted.*

RESULTS AND DISCUSSION

The maximum operation pressure was typically set at 350 bar, at around 400 bar the interface was damaged and leakages occurred. Injecting coumarin 440 and validating the performance in situ with a fluorescence microscope (Fig. 4), minimal

plate heights of 2.4-2.8 µm were obtained for both a 1 mm wide channel and a 150 µm wide channel (Fig. 5), with a slight effect of the turn on the performance.

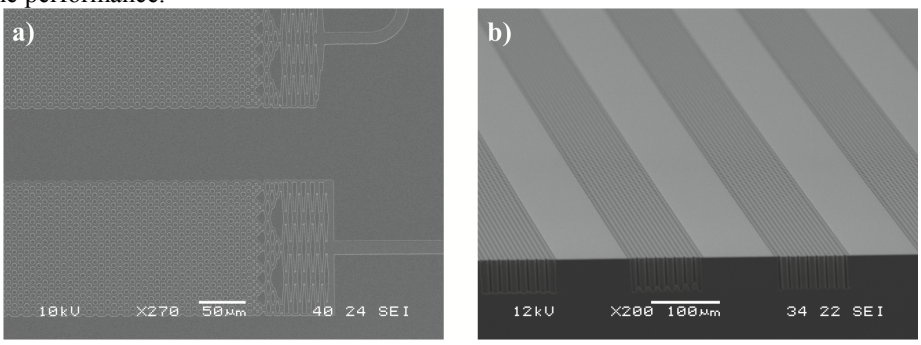

*Figure 2. SEM images of parallel channel tracks **a)** 150 µm wide. In the bottom right corner, the sample plug enters the channel through a low dispersion distributor structure. The track above, a turn is depicted where a transition to a 20 µm wide channel is accomplished by the same type of distributor structure, hence omitting a race track effect. **b)** Cross-section (channel width 100µm in this particular image).*

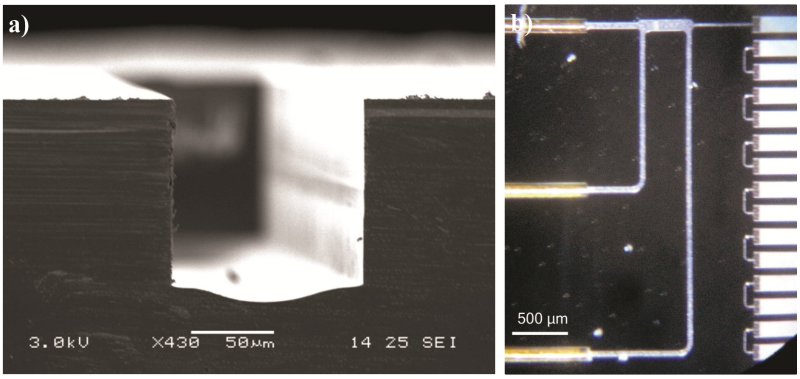

*Figure 3. **a)** SEM image of cross section of capillary insertion groove (without Borofloat top lid). **b)** Optical image of injection box. Left, the capillaries for mobile phase inlet and injection in- and outlet are depicted.*

Measuring at a channel length of 2.9 m (150 µm wide channel) at the optimal velocity, 1.07 10^6 plates were obtained, indicating an enormous separation potential. When interfacing the column with a commercial CAP LC instrument, larger volume channels are preferably used to minimize interfacing related dispersion [3].

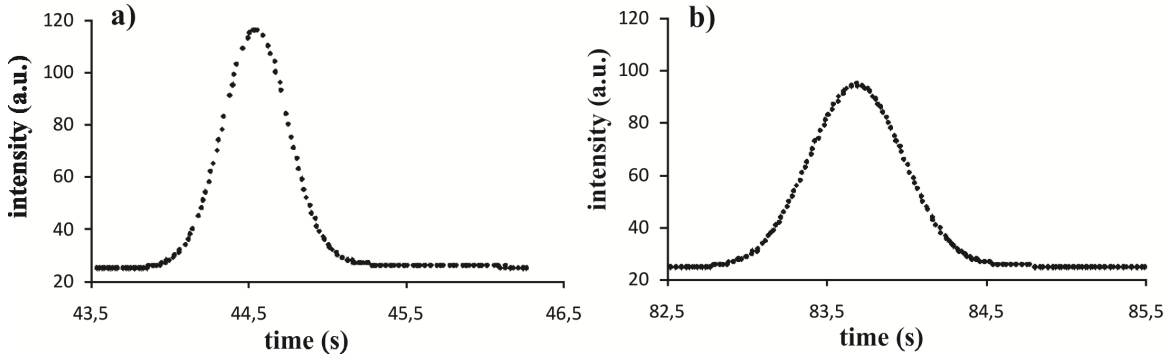

*Figure 4. Chromatograms of coumarin C40 in methanol (mobile phase velocity 2.55 mm/s) obtained by monitoring **a)** 10 cm and **b)** 20 cm downstream the injection (including one turn). The corresponding values for σ_t are 0.208 s and 0.294 s respectively, yielding a plate height of H=2.81 µm.*

Plugging a 1 mm wide, 50 µm deep (1.4 m long) channel into a commercial capillary LC instrument, excellent separations were obtained for steroid and tryptic digest separations (Fig. 6). Working in isocratic conditions, typically between 50,000 and 100,000 theoretical plates were obtained. It appeared that at the commercially used connection pieces jeopardized the overall performance seriously. For a 3 m column e.g., only typically 20 % of the plates obtained at the end of the column

were measured at the detector area after passing a connection piece. It is obvious that an important bottleneck in interfacing pillar array columns with commercial instrumentation requires the development connections with no stagnant zones.

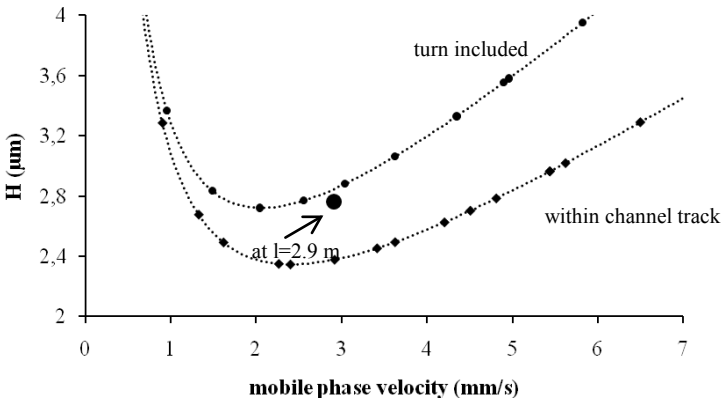

Figure 5. Experimental van Deemter curves and fits (dotted lines) of coumarin C40 in methanol obtained in a 150 µm wide channel determined over a 2 cm channel length without turn and over a 10 cm channel length including two turns . The big round symbol represents the plate height measured at a channel length of 2.9 m.

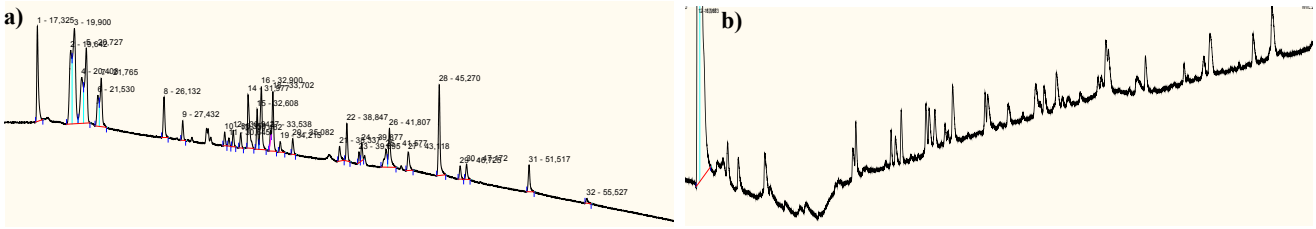

*Figure 6. Gradient separations in a 1.4 m long and 1 mm wide column detected with external injection (100 nl) and detection (volume detection cell: 3 nl) of **a)** 37 steroids (60 min run). Conditions: A: water +0.1 % TFA, B: acetonitrile, 0 to 100 % B in 60 min, flow rate: 2 µl/min, UV@210 nm, sample: 30 ppm each **b)** BSA tryptic digest (80 min run). B: acetonitrile/water 80/20 (0.4 % TFA), 0 to 95 % B in 200 min*

CONCLUSION

High pressure (350 bar) and high efficiency operation was demonstrated in a pillar array columns of up to 3 m long. Implementing distributors containing turns these long channels could be conceived with only a minor effect on the plate height. This resulted in a minimal plate height value of 2.8 µm, resulting in over a million plates measured at the end of a 3 m long channel. Preliminary experiments performed by coupling a 1.4 m column to a commercial detector and injection system, already indicate a large peak capacity. Future work will aim at minimizing dispersion at connection pieces.

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