PROTEIN DIGEST SEPARATIONS IN SILICON PILLAR ARRAYS CONFORMALLY COATED WITH POROUS SILICA

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ABSTRACT

The achievable separation performance of a 9 cm long pillar array channel containing 5 µm diameter silicon pillars (spacing 2.5 µm) cladded with a meso-porous silica layer with a thickness of 300 nm was investigated, interfacing the chip to a capillary LC instrument. The overall performance was evaluated by conducting gradient elution separations of digests of cytochrome c and bovine serum albumin. Peak capacities of up to 150 could be demonstrated.

KEYWORDS

Pillar array column, protein digest separation, porous layer integration, capillary LC, chip chromatography.

INTRODUCTION

A reduction of the critical dimensions of a flow device using microfabrication technology can result in improved chemical or analytical functions. For the specific case of liquid chromatography, the ability to create a packing with a high degree of order and a small flow resistance opens the road to produce highly permeable columns that are nearly completely devoid of the eddy-dispersion compromising the separation performance of the conventionally used packed bed columns. [1] The introduction of polymer and silica monolithic columns nearly two decades ago created an enormous momentum to develop novel packing structure for HPLC. With their high permeability and easy integration into longer columns, monoliths were identified as highly suitable columns to perform high-efficiency separations.

An important bottleneck of pillar array columns is their limited specific surface. [2] Attempting to mimic the traditional porous silica particles, with their well characterized and widely applied coating protocols, a sol-gel based deposition in the presence of pillars is an interesting approach. Coating at high values of domain size/inter-pillar distance, pillars can be uniformly coated with a meso-porous shell. [3]

EXPERIMENTAL

The pillar-array channel was lithographically patterned followed by dry etching steps to etch pillar (15.2 µm) and capillary fixation channels (130 µm), with 200 nm SiO_2 used as a hard mask, followed by anodic bonding to a 0.5 mm Pyrex substrate. Figure 1 shows an overall picture of the chip (before applying the porous-shell layer) and the connection tubing (Figure 1a), as well as some SEM pictures of one of the turns connecting two adjacent separation lanes (Figure 1b and 1c). These turns consist of a narrow connection channel of 10 µm (to minimize the volume of the turn), proceded and preceded by a flow distributor generally consisting of 5 µm × 63 µm diamond features, stretched perpendicular to the flow direction (spacing 2.3 µm). The aspect ratio of the transversally stretched pillars grows from around unity close to the separation bed to about a factor of 12 at the other end of the distributor.

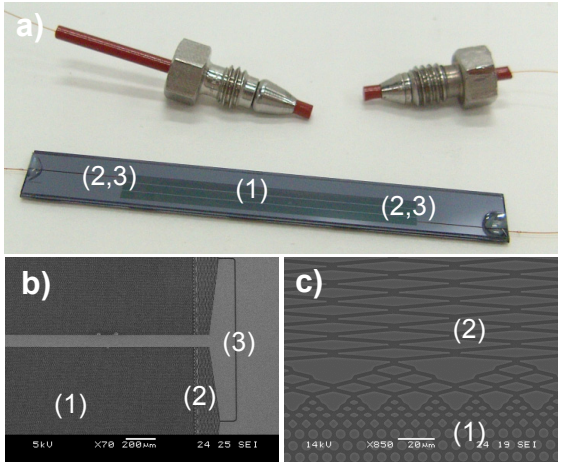

Figure 1 Optical picture of pillar array column containing three separation lanes of each 3 cm (depth 15.2 µm), the connection capillaries and the fittings, (1) is the pillar region, (2) is the distributor region, and (3) is a 10 µm wide turn channel b) overview (SEM) of pillar channel, distributor and turn channel c) close-up (SEM) of the flow distributor containing transition structures towards diamond pillars.

Capillaries (108 µm OD, 40 µm ID) were inserted in the grooves that appear after dicing and sealed by epoxy

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glue. A mixture of PEG (0.4 g), urea (1 g) and acetic acid (0.01 M, 10 ml) was inserted in the chip at 40 °C for 24 h leading to a confined spinodal decomposition. To create mesopores, the wet gel was heated in the oven at a rate of 1 °C per minute and left at 110 °C for 4 h, followed by pyrolysis step at 350 °C for 72 h.

Finally a 5 % C8 solution in toluene was flushed through the chip (2 d) to generate the reversed phase stationary phase. Separation experiments were performed with a capillary HPLC instrument (Dionex ultimate 3000).

RESULTS

The coating process for the applied pillar design, lead to a uniform coating along the entire channel length, see Figure 2 for a selected pillar region. One of the drawbacks mentioned by Detobel et al. was the occurrence of small pieces of monolith at the supply channels and around the injection zone. [3] At these relatively large channel dimensions, the small domain criterion required to obtain a conformal coating is not met, resulting in uncontrolled (and nonconformal) silica deposition. In the current design, these zones are omitted as the injection occurs off-chip and the supply capillary channels extend to the channel inlet and outlet. To avoid uncontrolled deposition in the connection capillaries, they were glued only at the border of the chip, so that after the cladding procedure, the capillary could be removed and replaced by a fresh one.

To enhance the detection sensitivity, large sample volumes (order of 1 µl) were loaded onto the column, by focusing the injected sample on the head of the column at the start of the gradient using initially a large water fraction. With the measured etch depth of 15.2 µm and a total channel width of 1 mm and the external porosity of 46%, the volume of the PAC channel equals about 0.6 µl (neglecting the internal pore volume), with the cross section corresponding to a cylindrical capillary with a diameter of 139 µm.

Using a 1 µl injection loop and a total of 45 cm connection tubing with 40 µm id, the system volume before the column can be estimated to be about 1.6 µl; the system volume after the column can be estimated to be 0.6 µl (25 cm connection tubing with 40 µm id glued to the chip, connected with a zero dead volume connection to 35 cm connection tubing with 20 µm id running through the UV-Vis flow cell). Due to the expected focusing of the analytes on top of the column, it is only the after-column volume that can be expected to contribute to the system band broadening.

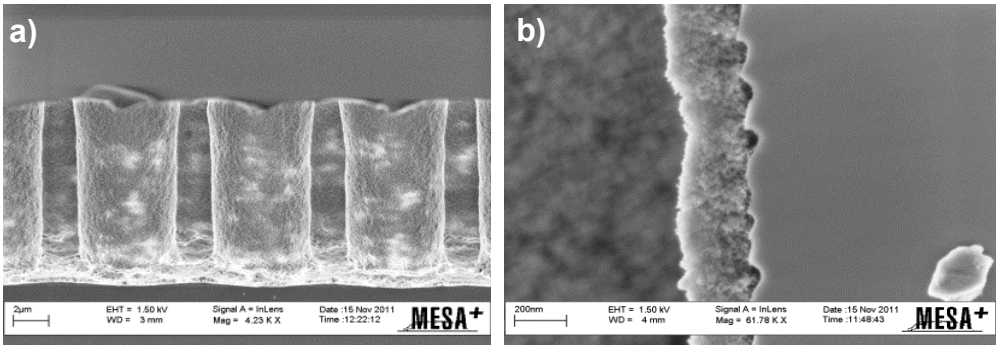

Figure 2. (a) Cross section (SEM) of the channel (b) zoom-in of porous layer at a cross section of pillar after cleaving the pillar. The layer thickness of the layer on the pillar mantle is on the order of 300 nm.

In Figure 3, a chromatogram of an albumin digest separation with excellent peak shapes is depicted. The peak shapes are very good and no fronting or tailing peaks are observed, indicating the absence of severe sidewall effects.

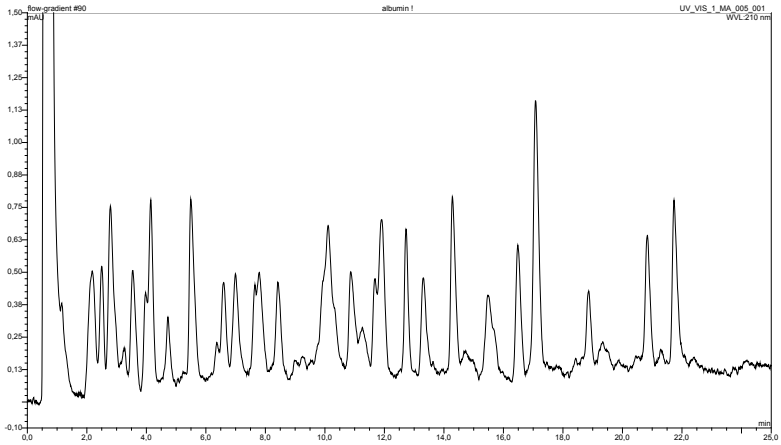

Figure 3. Albumin digest separations (0 to 50 % B in 30 min), sample: 1 µl, injected mass 1 µg, 4 µl/min, A: water (0.05 % TFA), B: 50/50 AN/H_20 (0.04 % TFA), 30 °C, 210 nm. The chromatogram spans 25 min.

The reproducibility was also studied by performing 25 gradient separations under identical conditions (Figure 4), resulting in retention time-based RSD values on the order of 5 %.

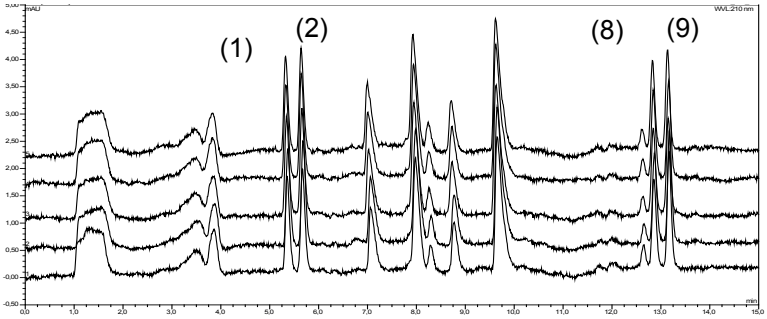

Figure 4. Repeated experiments (cytochrome c digest), 30 °C, A: water (0.05 % TFA), B: 50/50 AN/H20 (0.04 % TFA), 0-70 % B (7.5 min), 70-100 % B (0.1 min), 100 % B (2 min); 100-0% B (0.1 min), sample: 1 µl, injected mass 1 pmol, flow rate=4 µl/min. RSD's (n=25): (1) 5.4 % , (2) 4.8 %, (8) 4.8 %, (9) 4.3 % . The chromatogram spans 15 min.

The peak capacity was measured for a wide range of gradient times and flow rates (Figure 5), which is apparently hardly affected by the flow rate at identical gradient times. Peak capacities as high as 150 were obtained, which is an excellent performance, given the limited channel length (9 cm).

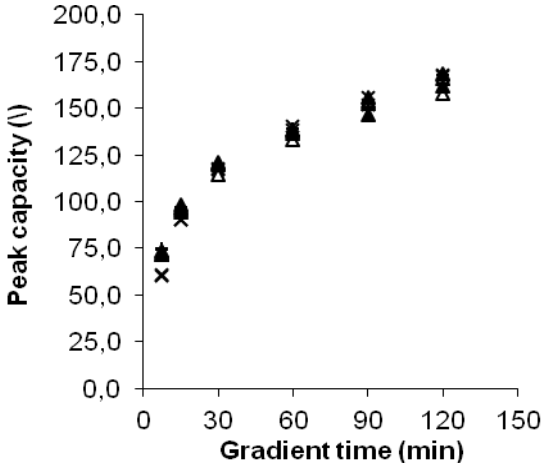

Figure 5. Peak capacities of cytochrome c digest (based on the average of peak 1, 2, 8 and 9) at 1, 2, 3 and 4 µl min in function of the gradient time.

Comparing this to an extrapolated performance based n a 5 cm × 0.1 mm fused core-particle column (2.7 µm, C18, Advanced Materials Technologies, Wilmington, USA), a stationary phase which is more comparable to the silica cladded pillars, operated at 60°C and with similar gradient times, where a peak capacity of Pc approximately 100 was obtained, the obtained result in the current study is similar. [4] Taking into account that the pillars used in the present study (after deposition of the porous layer) are as large as 5.6 µm (spacing 1.9 µm) and that the separations in the present study were conducted at room temperature, the obtained peak capacities are very satisfactory.

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