INTEGRATION OF UNIFORM POROUS SHELL LAYERS IN MICROFABRICATED PILLAR ARRAY COLUMNS BY ELECTROCHEMICAL ANODIZATION

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ABSTRACT

Electrochemical anodization has been applied to grow 300 nm thick porous shell layers in 5 µm diameter silicon pillars. These pillars are positioned in a triangular grid with 2.5 µm spacing to form a pillar array columns (PAC) for liquid chromatography. The uniformity of the porous layer was assessed by determining local plate heights along the column length, which appeared to be constant. Minimal plate heights between 4 to 6 µm were obtained at optimal flow rates and for different retention factors. Using the retention surface involved in retention as an indicator of the available surface, an increase in surface by a factor of 30 was found when comparing to non-porous columns. A commercial capillary LC instrument was modified to perform 2 nl sized injections which allowed more than 80% of the on-chip generated performance (expressed in theoretical plates) to be maintained when coupling a 1m long column to an external UV-detector. As the corresponding pressure drop for optimal operation is typically around 10 bar per m and the maximal pressure that can be applied is in the order of max of 350 bar, very long PACs, well capable of delivering much more than 1 million theoretical plates are within sight.

KEYWORDS

Microfluidics, Porous Silicon, Micro machining, Liquid chromatography, Micro pillar array column

INTRODUCTION

Packed columns with porous shell particles currently present an intensively studied and applied column format. The superior packing quality that these particles enableand the reduced C_m term contribution that goes along with partially porous support structures are indeed very appealing. This has allowed for a shift of the minimal achievable reduced plate heightof h_{min} ~2, a rule of thumb that was valid for decades, to a value of $h_{min} = 1.2$ [1]. A more extensive degree of order allows even smaller reduced plate heights, which can be practically achieved using micromachining technology developed for the microelectronics industry. During the last decade severalgroups have intensively worked on this topic implementing PACs, demonstrating reduced plate heights as low as $h_{min}=1$ for porous as well as non-porous pillars. To approach the same level of loadability as porous shell columns in silicon substrates, two approaches are at hand. First, a sol-gel deposition scheme similar as implemented in the silica monolith technology can be applied, which was developed by Detobel et al [2].
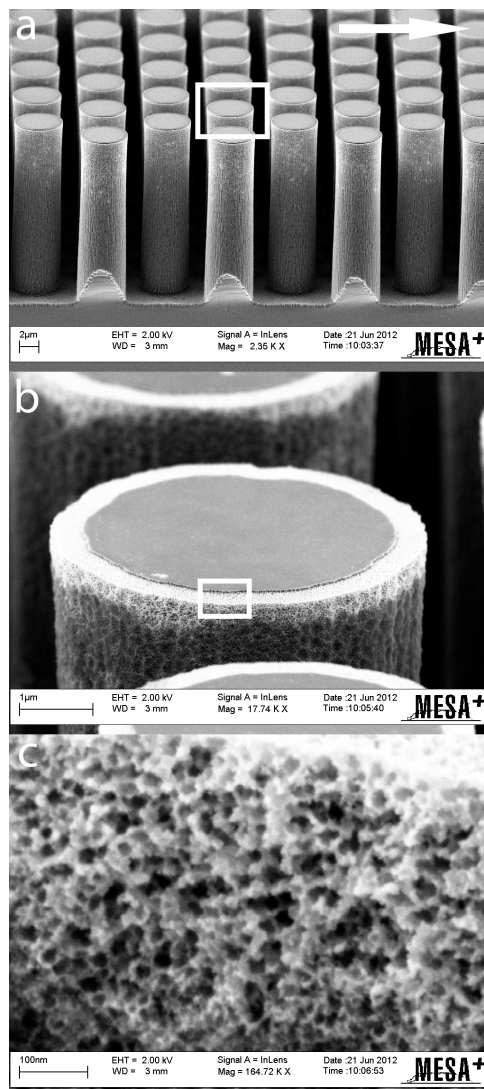

Figure 1: SEM images of the porous shell micro pillar array column (PAC) used throughout this study (a) The pillars are positioned on an equilateral triangle pattern with a distance of 2.5 µm between the pillars. The total diameter of the porous shell pillar is 5 µm, the height is 18 µm and the porous shell thickness is approximately 300nm. The arrow indicates the flow direction, the white box shows the used region for the higher magnification in the image below (b) One single pillar in detail, the green box shows the magnification for the image below (c) Zoom-in on the porous shell around the pillar. The pores are in the range of 30nm in diameter.

An alternative method to generate porous layers is electrochemical anodization, which effectuates in pores growing inwards the pillars, hence leaving the originally optimized flow profiles at the sidewall region unaltered [3]. Anodisation results obtained in this paper are shown in fig. 1. The technology was used earlier to study band broadening in the central part of 10 µm diameter porous pillar array channels, and was mainly interesting for theoretical considerations on the importance of the C_s intra-particle contribution of the C-term, a topic still very relevant with respect to the many debates regarding the real reason why porous shell particles perform so much better than what the (decreased) C_s contribution only would effectuate. In the meanwhile, different hurdles have been tackled in order to pave the way to make PACs the preferred column format to separate highly complex mixtures with HPLC. First, providing etched channels to insert and glue 110 µm capillaries into for a standard use of pressures of above 350 bar [4]. Second, the development of distributor features enables low-dispersion transitions at the channel inlet and outlet [5]. Third, the development of distributor-based turns makes it possible to generate long lengths with channels that have interesting cross section s for detection, i.e. that are typically at least a few 100 µm wide (e.g. a 300 µm wide and 18 µm deep[6].

So far, there has been a large discrepancy between the performance measured inside a pillar array with fluorescence microscopy and that measured with a commercial HPLC system. Measuring on-chip, dispersive effects related to sidewall-, turn- and interfacing can be excluded. In a practical separation, one is however only interested in what the detector records. When a sample band is injected and detected out of the chip, so far typically 80% of the performance was lost.

In the current contribution, electrochemical anodization was applied to create a porous shell layer of 300 nm thick on a folded channel containing 5 µm diameter pillars, and this in a vary uniform way. This channel was connected to a commercial capillary LC instrument which was reconfigured such that an automated on-chip injection of a few nl sample and of-chip (UV-Vis) detection (3 nl detection cell) could be performed in both isocratic and gradient mode, without suffering from jeopardizing coupling losses that generally occurs in classical capillary column operation. After studying the influence of the porous layer on retention, overloading and dispersion, the operation conditions to perform relatively ease separations in a short time frame, and more complex mixtures in longer time frame.

MICROFABRICATION

A highly doped p-type (100) Si wafer of 475 µm thickness and 10 cm diameter was patterned to define the pillars (18 µm deep) and the capillary channels (130 µm deep). The pillar-array columns (5 µm diameter pillars, inter-pillar distance 2.5 µm) were patterned using mid-UV photolithography (photoresist, Olin 907-12), followed by a dry etching step (Adixen AMS100DE, Alcatel Vacuum Technology, Culemborg, The Netherlands) to etch the 200 nm thick SiRN hard mask underneath. Next, the supply

channels were defined by mid-UV lithography, etching of the SiRN layer by a Bosch-type deep-reactive-ion etching step (AdixenAMS100SE) reaching a depth of 120 µm. After this, the resist was removed by oxygen plasma and nitric acid. The pillars defined in the SiRN mask (and also the already defined and partly etched capillary groove) were subsequently Bosch etched to a depth of 18 µm deep (and the capillary channel obtained a total depth of about 140 µm).

The substrate was then placed in a holder and immersed in 10% HF, while applying a constant current across the wafer for 10 and 15 min to respectively obtain a 300 and a 315 nm porous layer thickness. The microfluidic channels were subsequently sealed with a Pyrex wafer (thickness 0.5 mm), anodically bonded to the silicon using an EVG EV-501 wafer bonder (EV Group Inc., Schaerding, Austria). Next, the chip was diced (100 µm deep) from both sides of the wafer and subsequently cleaved, exposing the grooves wherein the interfacing capillaries fit (108 µm OD and 40 µm ID). Subsequently, the capillaries were inserted in the grooves and sealed by epoxy glue. The coating was C8 (octyldimethyl silane) and HMDS as end-capping agent.

INJECTION AND DETECTION

The sample injection was performed using an automated valve system, controlled with an in-house written C++ program, as already described in [9]. For the mobile phase propagation an Agilent 1100 series nano pump (Agilent Technologies, Waldbronn, Deutschland GmbH) was used. During the sample injection step, the automated valve system ensures that the inlet and the outlet of the mobile phase circuit were closed. During the subsequent sample separation, the inlet and the outlet of the sample injection circuit were diverted to a high flow resistance capillary, allowing for the existence of a small leakage flow and thus avoiding tailing. A Hg-vapour lamp was used to excite the fluorescent dye in the UV. The peaks were visualized using an air-cooled CCD fluorescence camera (Hamamatsu Photonics K.K., Japan). The peak intensity profiles were subsequently analyzed using the accompanying Simple PCI® image analysis software. On-column chromatograms were obtained by averaging a row of pixels and plotting its values in function of time. For the determination of the peak widths and plate heights, the peak fluorescence intensity was averaged across the channel width. The thus obtained axial concentration distribution was subsequently fitted with a Gaussian function using Matlab®. H is then obtained as $(\sigma_{x,e}^2 - \sigma_{x,0}^2)/\Delta x$, wherein $\sigma_{x,e}$ and $\sigma_{x,0}$ are the variance of the injected band at the end and the initial position and wherein Δx is the distance between both positions.

RESULTS AND DISCUSSION

To maintain the advantage of the ordered nature of the non-porous pillar array, it is critical that the added surface is uniformly distributed along the height of the channel. Under the applied anodization conditions, the thickness of the porous layer was 300nm for the 10min anodisation (fig. 1b).

Besides the large uniformity that can be attained regardless of the surface to be processed, a strength of anodization is that the pore size can be easily altered by varying the electrical potential. For the current study a sufficiently large pore size was aimed for, in order to make the format also of direct interest for the separation of relatively large components. A pore size in the order of 30 nm was achieved under the current conditions (Fig. 1c). To study the dispersion and retention characteristics of the channel, a mixture of 4 coumarins with varying retention properties was injected (Fig. 2). From the CCD image, a band width of 200 µm can be estimated for the injected band (Fig. 2a), corresponding to approximately 2 nl of sample. At 3.5 mm distance in the column, the bands are baseline separated, as can be seen in the chromatogram that was established by plotting the average intensity at that position in function of time (Fig. 2b).
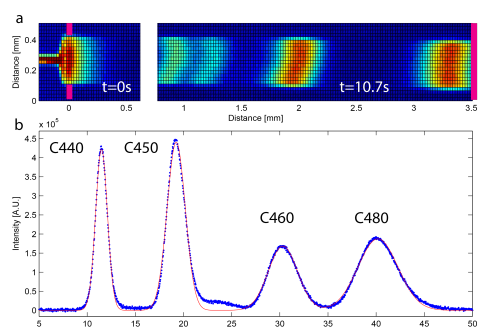

Figure 2: Chromatogram showing a separation of four coumarines (C480 = 0.40mM, C460 = 0.50mM, C450 = 0.17mM, C440 = 0.20mM). The chromatogram was recorded at 3.5 mm downstream the injection in a 55/45% methanol/water mobile phase mixture at a temperature of 25°C. At a linear speed of 0.31mm/s, C440 (the unretained component), C450 (k'=0.67), C460 (k'=1.64) and C480 (k'=2.50) yielded a plate height of H=5.6µm, 6.9µm, 8.1µm and 9.0µm. a) Camera image of the separation. The broken magenta line indicates the start of the column, the other magenta line indicates the monitor line where H was determined b) The blue dots represent the actual fluorescent intensities measured at the monitor line as function of time. The red line is a sum of four Gaussian peaks fit using Matlab®. The fitting was used to calculate k' and H values.

A major hurdle in the use of the miniaturized columns (including capillary columns) is interfacing them with the outer world. In recent work, we have developed a glue protocol so that operation pressures of at least 350 bar can be applied to the channel. This allows direct implementation of the column in a capillary LC instrument, using the automated injection system of the device to inject samples with volumes between 100 nl and 1 µl. For a typical pillar array column, this injection volume is too large as the channel volume is in the same order, which has a detrimental influence on the attainable peak capacity. By reconfiguring the capillary LC instrument, the runs were programmed to enable an automated on-chip injection. This was performed on a 25 cm long channel (volume 1.6 µl), resulting in a H_{min} value

of 12 µm for a. Considering that the capillary volume between the chip and the detection cell 150 nl (11 % of the separation channel volume) was, it is of not surprising that a significant portion of the performance was lost. Increasing the channel length to 1m and decreasing the ratio of the column volume to the external volume to 3 %, the on-chip and off-chip performance could be brought to a much more acceptable ratio of on-chip versus off-chip performance (75 % for a non-retained component and 50 % for a retained component).

In order to verify whether the additional dispersion is merely related to the coupling to external devices, a number of on-chip plate height values were determined by monitoring a fluorescent tracer dye inside the pillar bed (Fig. 3). The homogenity of the porous layer over the entire column length was evaluated by monitoring a single peak over a total length of 250 mm, but at small distance intervals. The plate height is found to be quite constant over this distance which is indicative for a high degree of uniformity in porous layer thickness.
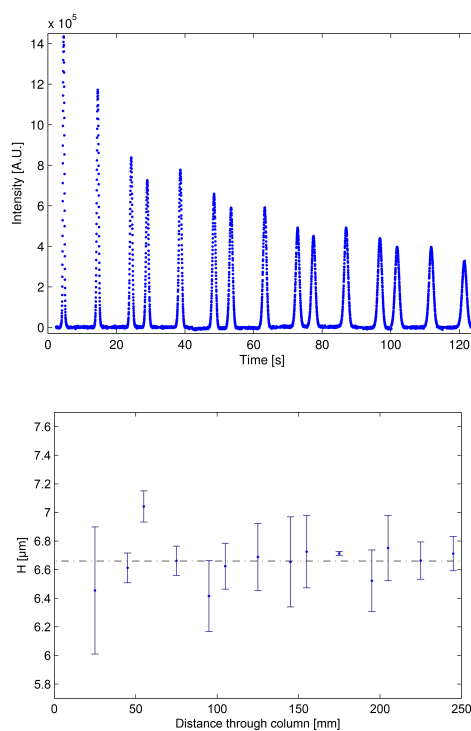

Figure 3: a) Local chromatograms of a non-retained C480 sample band obtained at different residence times in the column (100 % methanol, 25 °C, 1mM, 300 nm thick layer, 2.1 mm/s) b) The corresponding plate heights of the peaks obtained at the residence times in a) (n=3, 95% confidence bounds error bars). The overall average plate height for that speed is 6.7µm (black dashed line).

The increase in surface area can be estimated by comparing the retention factors before and after anodization at identical mobile phase compositions (Fig. 6). An increase in k'-value of 25 times can be observed when comparing non-porous columns to columns with a 300nm thick porous shell layer. The fact that the slope is different, is related to the different accessibility of the surfaces in the non-porous and porous columns.
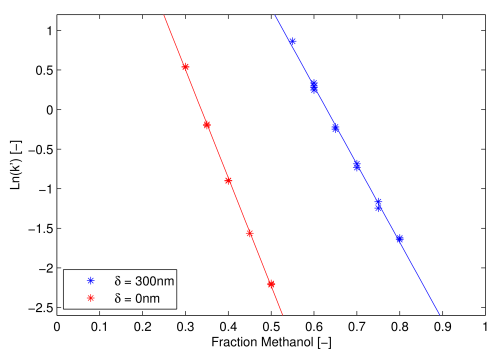
Figure 4: Retention coefficient of C480 (C440 used as non-retained marker) plotted as a function of the fraction methanol in the mobile phase at 25°C (Base 10 logarithm) for a PAC with (dashed line) and without (full line) a 300 nm thick porous layer. The concentration of C440 and C480 were 0.20 and 0.40 mM respectively, the injection volume was approx. 2 nl.

The required pressure to operate the columns at their optimal linear velocity ranges from 10 bar/m for a retained to 50 bar/m for a non-retained component. The peak capacity of the channel was subsequently determined at different flow rates, revealing that a maximum of around 200 could be achieved in a 25 cm long channel (based on an average of 9 components with varying retention). Striking to note is that the pressure required to obtain this maximal peak capacity was only 10 bar.

In Fig. 5a, an isocratic separation of 9 components is shown, where all components are baseline separated within 4 min. This demonstrates that the column is also suitable for relatively fast routine separations of a limited number of components. Isocratic operation of capillary format columns is extremely challenging and israrely described in the literature because the interfacing and injection related dispersion typically ruins the separation.

The peak shape quality and separation performances presented here are to a large extent achieved by injecting on-chip and implementing low-dispersion connections. Performing the separation under gradient conditions, again excellent shapes were obtained. Under these conditions, a peak capacity of 71 was obtained in 10 min. Working under optimal conditions, a peak capacity of 198 was obtained in 160 min.

Another important property of these columns is that the accompanying pressure to work under optimal conditions is only 10 bar, which creates possibilities towards different solvent propagation approaches. Obviously low pressure pumping devices are much cheaper, which could increase the accessibility of HPLC systems at unusal working conditions (i.e. outside a laboratory environment). Given the advance in the development of miniaturized HPLC pumps, with micropumpsthat can deliver more than 15 bar based on electro-osmotic flow pumping, battery driven high performance LC devices might soon be integrated in a hand-held HPLC suitcase.
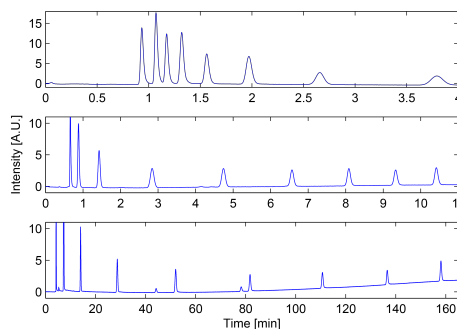

*Figure 5: Chromatograms showing 9 components (each 100 ppm)for three different conditions: **a)** Isocratic 100bar 50%water-50% acetonitrile:(uracil: k'=0 / 47.6µm,acetanilide: not present, acetophenone:k'=0.14 / 36.2µm, propiophenone: k'=0.26 / 35.0µm, butyrophenone: k'=0.41 / 37.4µm, valerophenone: k'=0.67 / 39.1µm, pentyl-phenyl keton: k'=1.1 / 44.0µm, heptaphenone: k'= 1.8 / 47.6µm, octaphenone: k'=3.6 / 53.5µm)**b)** 200bar, gradient as fig.8 **c)** max PC 10bar, gradient mode.*

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REFERENCES

[1] DeStefano J.J.,"Performance characteristics of new superficially porous particles",J. Chromatogr. A, 2012, vol. 1258, pp. 76–83.

[2] De Malsche W.,"Separations using a porous-shell pillar array column on a capillary LC instrument", J. Sep. Sci*.,* 2012, vol. 35, pp. 2010-2017.

[3] De Malsche W.,"Integration of porous layers in ordered pillar arrays for liquid chromatography", Lab Chip 2007, vol. 7, pp. 1705-1711.

[4] De Malsche W.,"Capillary liquid chromatography separations using non-porous pillar array columns", 2012, J. Chromatogr. A, vol. 1230, pp. 41-47

[5] Vangelooven J.,"Design and evaluation of flow distributors for microfabricated pillar array columns", Lab Chip, 2010, vol. 10, I. 3, pp. 349-356

[6] Cabooter D.,"Detailed characterisation of the flow resistance of commercial sub-2 mu m reversed-phase columns", J. Chromatogr. A, 2008, vol. 1178, pp. 108–117.

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