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Highlights

- A new chromatographic column format is proposed
- A microfabricated silicon template is used to ensure perfect order of the particles
- Assembly of the particles in template could be realized using PDMS-assisted rubbing
- Validation of the concept using Computational Fluid Dynamics
- Small imperfections prevent chromatographic tests

Structured Microgroove Columns as a Potential Solution to Obtain Perfectly Ordered Particle Beds

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Abstract

We report on a novel concept to produce ordered beds of spherical particles in a suitable format for liquid chromatography. In this concept, spherical particles are either positioned individually (single-layer column) or stacked (multi-layer column) in micromachined pockets that form an interconnected array of micro-grooves acting as a perfectly ordered chromatographic column. As a first step towards realizing this concept, we report on the breakthrough we realized by obtaining a solution to uniformly fill the micro-groove arrays with spherical particles. We show this can be achieved in a few sweeps using a dedicated rubbing approach wherein a particle suspension is manually rubbed over a silicon chip. In addition, numerical calculations of the dispersion in the newly introduced column format have been carried out and demonstrate the combined advantage of order and reduced flow resistance the newly proposed concept has over the conventional packed bed. For fully-porous particles and a zone retention factor of k'' = 2, the h_{min} decreases from h_{min} = 1.9 for the best possible packed bed column to around h_{min} =1.0 for the microgroove array, while the interstitial velocity-based separation impedance E_i (a direct measure for the required analysis time) decreases from 1450 to 200. The next steps will focus on the removal of occasional particles remaining on the sides of the micro-pockets, the addition of a cover substrate to seal the column and the subsequent conduction of actual chromatographic separations.

Keywords: Liquid chromatography; Column formats; Nano/microfabrication; Kinetic plots; Particle assembly

1. Introduction

The current standard in High Performance Liquid Chromatography (HPLC) is based on the use of cylindrical stainless steel columns or fused-silica based capillaries packed with a slurry of spherical microparticles [1–8].

However, extensive computer simulations and theoretical studies have shown that the separation performance of these columns, measured in terms of the number of theoretical plates (*N*) per meter or in terms of the plate height *H*, is significantly compromised by the random nature and overall heterogeneity of the packing structure in the slurry-packed cylindrical columns [9–14]. Simulations have shown that severely increased separation performances can be obtained when the particle beds would no longer be randomly packed, but perfectly ordered. More specifically, minimal reduced plate heights h_{min} of 0.64 for a non-retained compound or 0.84 for a compound with retention factor k'' = 2 were obtained in silico [12]. This is significantly less compared to the current state-of-the-art packed bed columns where h_{min} is typically around or slightly below 2 in the case of highly monodisperse fully-porous particles and around 1.5 in the case of core-shell particles [15,16].

This insight has, in the past few years, led to the proposal of a number of different approaches to produce perfectly ordered chromatographic columns. One suggested approach is the use of 3D-printing. In practice however, the use of additive manufacturing for fabricating chromatographic columns is still in its infancy and has currently only been demonstrated for preparative chromatography [17]. The same holds for the multi-capillary column with diffusive bridging concept recently introduced by Parmentier [18]. One other approach, based on using photolithography and plasma etching to produce silicon-based micro-pillar array columns has made it to commercialization in the area of nano-LC for proteomics[8,19–23]. Although the induced order and the enlarged flow resistance of the micro-pillar array columns indeed lead to groundbreaking performances, these columns are silicon-based and currently offer a very limited choice of stationary phases. More specifically, the micro-pillar array columns do at present not provide the wide variety of stationary

phases offered by the commercially available silica-based particles whose retention properties have been optimized to perfection for decades by a vast group of scientists in academia and industry.

Considering the enormous wealth of selectivities offered by these particles, and given the aforementioned advantage perfectly ordered columns would have in terms of separation efficiency, there is a clear motivation to conceive a column format wherein conventional HPLC particles could be packed in a more orderly fashion than what is possible in the currently used randomly packed bed columns.

To this end, it is proposed here to arrange chromatography particles in micro-structured groove networks produced by micromachining silicon wafers as a generic new type of column format. In the present contribution, we show how such micro-groove structures can be filled in a few 10's of seconds with monodisperse 10 µm silica particles using an in-house developed suspension application and rubbing procedure. In addition, we also discuss the theoretical separation efficiency and kinetic performance potential of the proposed concept.

2. Experimental section

Micromachined silicon devices composed of an array of interconnected microgroove structures with cylindrical pockets with a diameter of 12 µm (designed to receive individual 10 µm silica particles) have been fabricated. These pockets formed the unit cell in micro-groove columns with different connectivity patterns (cf. Fig. S-1 of the Supplementary Material (SM)). Each column had a length of 60 mm and a width of 7 mm, and had a bifurcating distributor (cf. Fig. 1A) at both ends.

2.1. Fabrication of template

The channel networks were fabricated on a standard 4-inch silicon wafer (one side polished, (100), p-type) by first transferring the desired pattern through standard photoresist lithography [24] and subsequently using a deep reactive-ion etching (DRIE) process to transfer the pattern into the bulk of

silicon wafer. For the latter, a three-step DRIE process was used to achieve nearly vertical sidewalls with a scallop size of 30 nm [25].

The etching process used a combination of C_4F_8 and SF_6 gases and was implemented on a PlasmaPro 100 Estrelas machine (Oxford Instruments). The pattern was etched 12–13 µm deep into the silicon. Prior to the particle filling experiments, the substrates were thoroughly cleaned by O_2 plasma (Tepla 360) to remove fluorocarbon and photoresist residues. A final cleaning with HNO_3 was performed to remove any remaining organic residues.

2.2. Filling of the template with particles

The procedure for filling the silicon-based template with particles was adapted from earlier work done in-house on the manual rubbing of particle slurries over micro-structured surfaces inspired by the pioneering work performed by the Yoon group [26], where the use of PDMS rubbing for template-assisted particle assembly was described for the first time (for dry conditions). Here, 10 µm calibrated, monodisperse silica particles (MicroParticles GmbH, Germany) were first suspended in filtered 2-propanol at a concentration of 50 mg/mL. Two distinct strategies for presenting particles to the template were investigated. The first strategy consisted of using a micropipette to uniformly spread out 10 µL of the sonicated particle suspension along the length of the columns. The second presentation method involved the construction of a fluidic cell on top of the columns, whereafter 20 µL of the sonicated particle suspension was presented at the inlet of the fluidic cell using a micropipette. Due to a driving capillary suction force, the particle solution is sucked inside the fluidic cell, and the particles get distributed over the templated surface, as reported by Yin et al. [27]. Note that both particle presentation methods gave similar results in the present study. After supplying the particles on the templated surface, the assembly process was continued by performing a couple of rubbing strokes using a PDMS slab (Sylgard 184 mixed in a 10:1 (monomer:crosslinker) ratio, Dow Corning/VWR). The PDMS slabs used in this work were profiled, having trenches of 50 µm deep and wide, spaced 50 µm from each other. This allowed for further optimization of the silica particle assembly, as these profiled PDMS slabs simultaneously allowed excessive particles to be evacuated

through the PDMS slab's trenches. Finally, the quality of the assembly was assessed using Scanning Electron Microscopy (JEOL JCM-6400, Zaventem, Belgium). For more details of the particle assembly process and the patterning of PDMS slabs, the interested reader is referred to the "wet PDMS rubbing" assembly method already described by Verloy et al. [28].

2.3. Numerical methods

All simulations were performed using COMSOL Multiphysics 5.5. First, the unit cell of the desired geometry was designed using the COMSOL CAD-module (cf. Fig. S-2 in the SM). A periodic boundary condition was implemented on the fluidic in- and outlet of the unit cell. Next, a computational mesh was generated. The vertical symmetry of the problem was used to minimize the computational load. The mesh consisted of a combination of tetrahedra, pyramids and prisms, totalling 71 528 elements. Hereafter, the velocity field of the fluid zone was obtained using the AMG GMRES method in COMSOL to iteratively solve the Navier-Stokes equations. Based on this velocity field and a range of dimensionless velocities, the so-called B-field from the Brenner dispersion theory [29] was calculated. This led to the effective axial dispersion coefficient D_{ax} [30], from which the reduced plate height could be calculated. The appropriateness of the Brenner dispersion theory to calculate chromatographic data was validated in-house, and resulted in a perfect agreement of the obtained plate height values compared to those described in literature CFD studies [9].

3. Description and results

Fig. 1A instantaneously shows the general idea of the proposed concept, wherein a chromatographic column would consist of an array of interconnected cylindrical pockets having a depth ranging between one and say 50 particle diameters deep and a diameter ranging between one and say 5 particle diameters wide (upper limits only given as an order of magnitude). In the specific layout represented in Fig. 1A, each cylindrical pocket has been designed to receive precisely one spherical particle, ensuring that all particles are in a fixed, well-defined location in the column. In another possible layout (cf. Fig. 1B), single particles would be stacked on top of each other with the purpose

of enlarging the column's cross-section to enable larger flow rates. Indeed, requiring silicon micromachining for its fabrication, the micro-groove column concept suffers from a similar limitation as the micro-pillar array columns: with a production cost that is directly determined by the column's footprint on a silicon wafer(=the starting material for the column's production), and only weakly depends on the depth of the machined structures, the economically most viable strategy to enable larger flow rates is by designing columns that have the largest possible depth. However, this option can also not be exploited unlimitedly. In general, it is considered that pocket depth over diameter aspect ratios of 10:1 are relatively straightforward to achieve without suffering from a significant tapering of the etched grooves along the depth axis, while aspect ratios up to 30:1 require some dedicated optimization to prevent undesirable tapering while aspect ratios up to 50:1 are generally considered as the upper limit of the DRIE technology. Considering a pocket diameter of 5 µm, the aforementioned implies micro-groove columns could be made 50 to 250 µm deep. Here another significant advantage (next to the ability to use commercial HPLC particles) this new concept has compared to micro-pillar array columns emerges. Indeed, the critical lateral distance in the latter case is the inter-pillar distance. This is only of the order of 1 µm, such that the aspect-ratio restrictions currently only allow for micro-pillar array columns that are 10 to 50 µm deep.

The micro-pockets can be interconnected in a wide variety of channel patterns, some of which are represented in Fig. 1C (see Fig. S-1 for an overview of the four different layouts considered in the present study). The preferred channel pattern should reflect the best possible compromise between a maximal degree of transversal mixing (requiring transversally stretched and hence strongly meandering micro-groove channels) to counter the inevitable channel-to-channel velocity differences and a minimal pressure-drop (requiring straight running channels) [31].

To assess the theoretical potential of the concept, the pressure-drop and band-broadening characteristics have been determined using computational fluid dynamics and the Brenner method. Knowing from earlier work on pillar array columns that the kinetic performance of columns

composed of a parallel bundle of interconnected channels is quasi-independent of the degree of channel tortuosity [31], and invoking Brenner's dispersion theory [29,30], the unit cell can be limited to a single pocket (Fig. S-2). The red and green curves in Fig. 2A show the computed evolution of the dimensionless plate height (h) with the reduced velocity (ν) for two zone retention factors for the fully-porous particle case. Compared to the random packed bed case (represented by a set of van Deemter parameters A=1, B=2, C=0.1 leading to the best possible h_{min} (h_{min}=1.9) for fully-porous particles [32]) the gain obtained from the increased order is obvious. The selected example (a 1 µm particle in a 1.15 µm diameter wide and 1.25 µm deep pocket) leads to a relatively large external porosity (ε=61.6 %) and thus still has relatively large mobile phase mass transfer distances. Consequently, the h_{min} does not go as deep as for example obtained for the ordered particle bed geometry considered in [9], however still lies slightly below h_{min} = 1, which could be considered as the hallmark value for perfectly ordered beds (see, e.g. earlier simulation results in [9–14]) and is about a factor of two better than in the random packed bed. Because of the relatively high external porosity, the flow resistance is considerably lower than in a randomly packed bed. Computing the interstitial velocity-based permeability (K_{vi}) and its corresponding flow resistance ϕ_i as indicated in the description of Fig. S-2 in the SM, a flow resistance of ϕ_i =132 is obtained. This is about a factor of 3 smaller than the ϕ_i in a randomly packed bed, where typically ϕ_i = 400 [33].

The latter obviously constitutes a huge additional advantage when comparing the two systems in a kinetic plot [34,35], allowing an impartial assessment of the true speed and efficiency potential of chromatographic systems with different geometry. As can be noted directly from the dashed Knox and Saleem-limit lines added to Fig. 2B, micro-groove columns would represent a gain in separation speed of a whole order of magnitude. In the practically most relevant range of plate numbers of N=20 to 40,000, optimal particle diameters would be 2 µm, or even smaller.

When contemplating the use of micro-groove packed columns, the first hurdle to overcome is the filling of the grooves. To tackle this problem, we have adapted an in-house developed wet manual rubbing technique, for which the reader is referred to the experimental section.

With this method, we can now reliably fill micro-groove columns containing around 1 million pockets (width = 2 mm, length = 5 cm) with an empty pocket ratio on the order of 0.1%. Fig. 3 shows some examples of the finally obtained structures, including the case of multi-particle filled pockets. Zoomed-out pictures, demonstrating the capability to address mm-large areas are shown in the SM (Fig. S-5). Obviously, when the grooves are filled with particles having a broad size distribution flow patterns will be more heterogeneous and thus lead to lower performance. However, the high frequency of radial contact points between adjacent flow paths will help keeping equal pressures to counter this effect." The main current nuisance is the occasional presence of hard-to-remove particles remaining on the chip surface after filling and cleaning with the PDMS slab (Fig. 3D). Although these occur in very low numbers (10 to 20 extra particles on a total of 1 million), they constitute an important problem when trying to add a cover plate to seal the micro-groove channels. Their presence can lead to imperfect bonding (and hence create leak flow paths) or even to breakage of the cover plate since any conceivable bonding process requires the application of a strong downward pressing force. Occasionally, also some small PDMS debris originating from the employed rubbing slabs can stick very strongly to the chip surface or particles. This debris originates from the sharp edges of the micropockets in combination with imperfections around the edges (cutting lines) of the PDMS slab. More work, using very precisely shaped and cut PDMS slabs, needs to be carried out to remove these last impediments.

4. Conclusion

We have proposed a generic concept wherein spherical silica particles can be arranged in ordered micro-groove networks such that disorder is limited to either the single particle level (cf. Figs. 3A-B) or to the level of a few particles at most (case wherein pocket diameter spans 2-5 particle diameters

shown in Fig. 3C). Due to their interconnected design, the microgrooves also have an inherent mechanism for the correction of velocity differences among the different branches. The latter will also be important when the grooves are filled with particles having a broad size distribution, in which case flow patterns will be more heterogeneous and thus lead to lower performance. Because of the increased order, significantly lower plate heights (order h_{min} = 1 can be expected). Combining this with the lower flow resistance (originating from the fact that the inescapable vicinity of the column leads to higher external porosities than those prevailing in random packed beds), significant gains in separation speed (order of factor 10 when neglecting groove-to-groove packing density differences). The format also has a small disadvantage in that, given the volumetric relationships, the phase ratio (volume of particles vs mobile zone volume) will be some 30 to 50% smaller than in packed columns such that micro-groove columns will have a lower mass loadability. They will also require slightly weaker mobile phases to keep the same retention times as in a packed column, but this in itself is not problematic. Given the strong dependency of the flow resistance and the phase ratio on the external porosity, in turn determined by the ratio of the pocket size dimensions and the particle diameter, this ratio is an important design parameter to find optimal compromises between efficiency, speed and mass loadability

At present, we have brought this idea to the level where micro-groove columns can be repeatably filled with an empty pocket ratio of 0.1% and an excess particle ratio of 0.001%. Despite their low number, these excess particles disturb the bonding of a glass cover on the channels. Solving this problem is hence the next challenge in realizing this concept. Other challenges may emerge when this concept is applied to smaller particles and for particles with larger degrees of polydispersity, as well as the filling of grooves with a multi-particle depth running. Future work should also involve optimizing the groove network design (degree of connectivity and tortuosity of the grooves, optimal diameter pockets, optimal transition between pockets,…).

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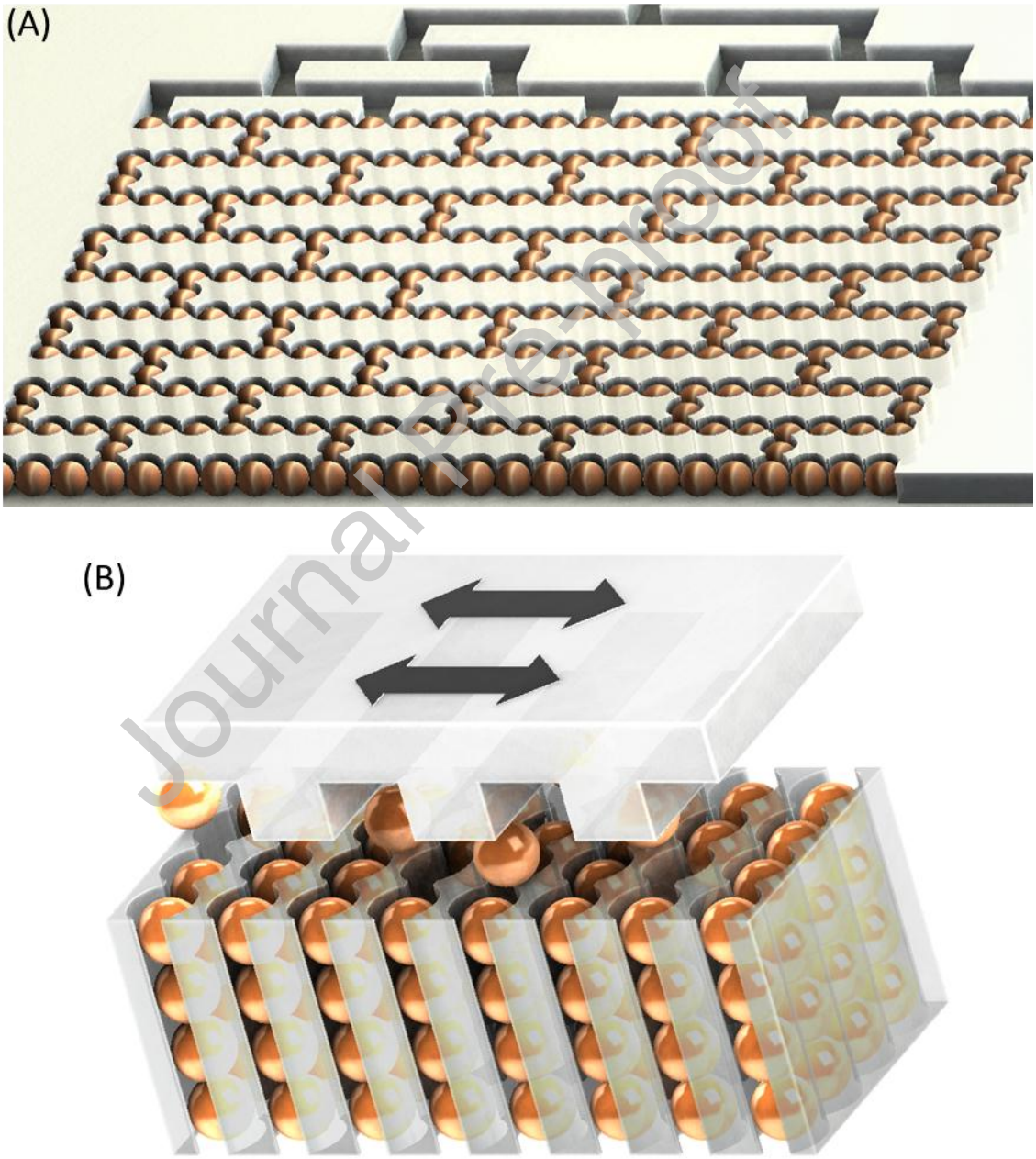
Figure Captions

Figure 1. (A) Conceptual image showing the principle underlying the new concept wherein a micro-groove template is used to perfectly organize arrays of chromatographic particles. **(B)** Schematic representation of the filling-by-rubbing methodology wherein a profiled PDMS slab (light grey) is used to sweep micro-sphere particles (blue) into the silicon template (dark grey). **(C, D)** SEM images of empty templates show two possible micro-groove network configurations.



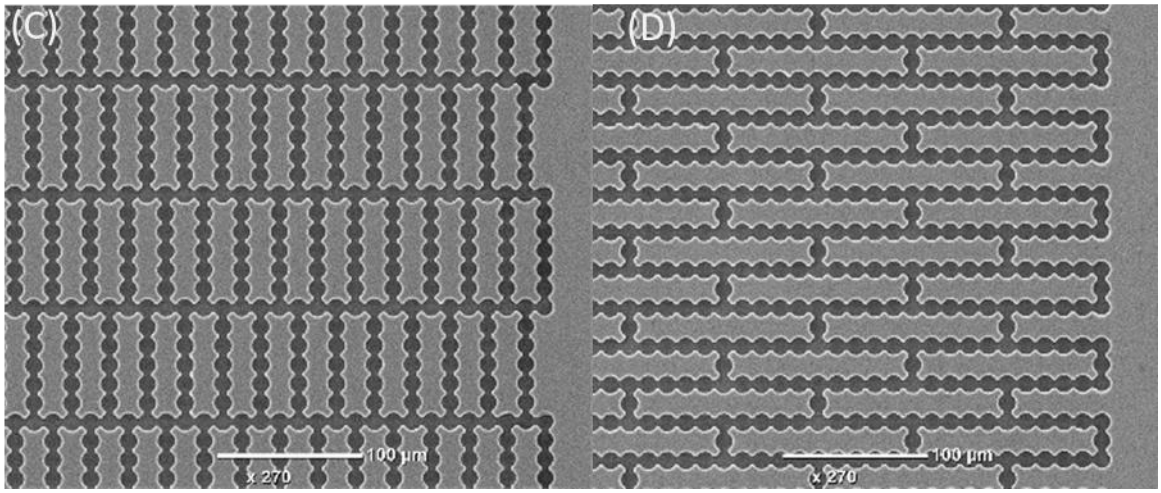
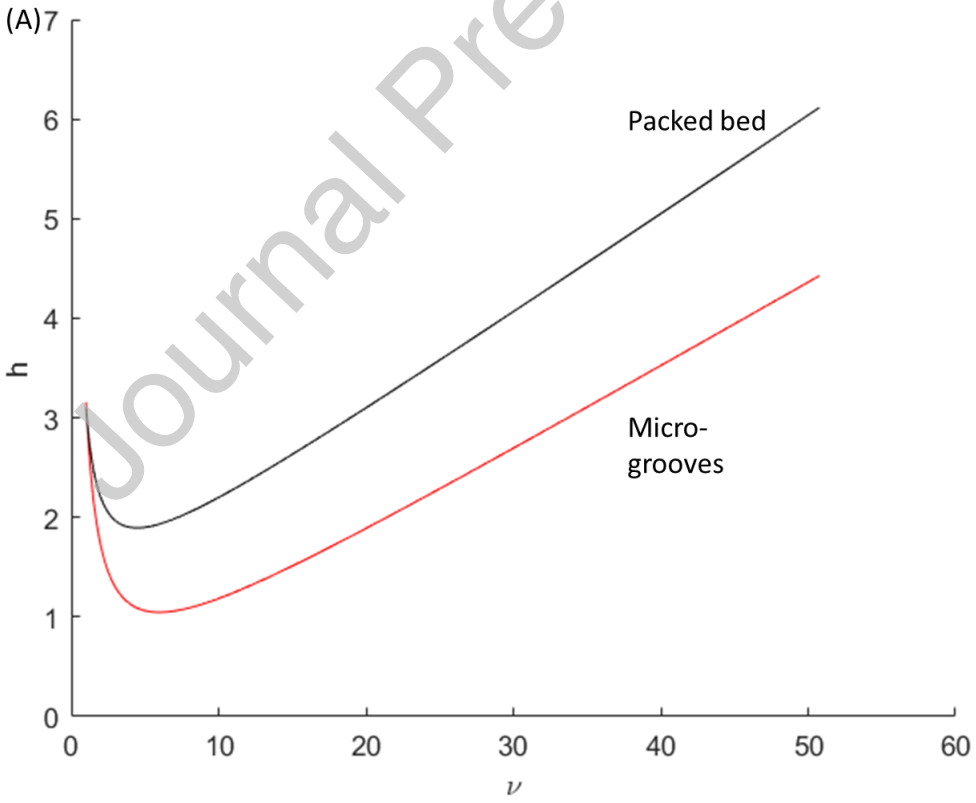


Figure 2. (A) Van Deemter curve showing the reduced plate height versus the reduced velocity ν (=$u.d_{part}/D_{mol}$) for a typical well-packed fully porous particle bed column (black) compared to that predicted for a grooves network column (red).**(B)** Kinetic plots of t_0 versus the theoretical plate count for a particle bed column (black) and grooves network columns for k"=2 (red) and k"=5 (green). Kinetic plots shown for two different particle sizes (d_{part} =2 µm and 5 µm). The dashed lines represent the Knox and Saleem limit for a packed bed column (black) and a grooves network column with k"=2 (red).



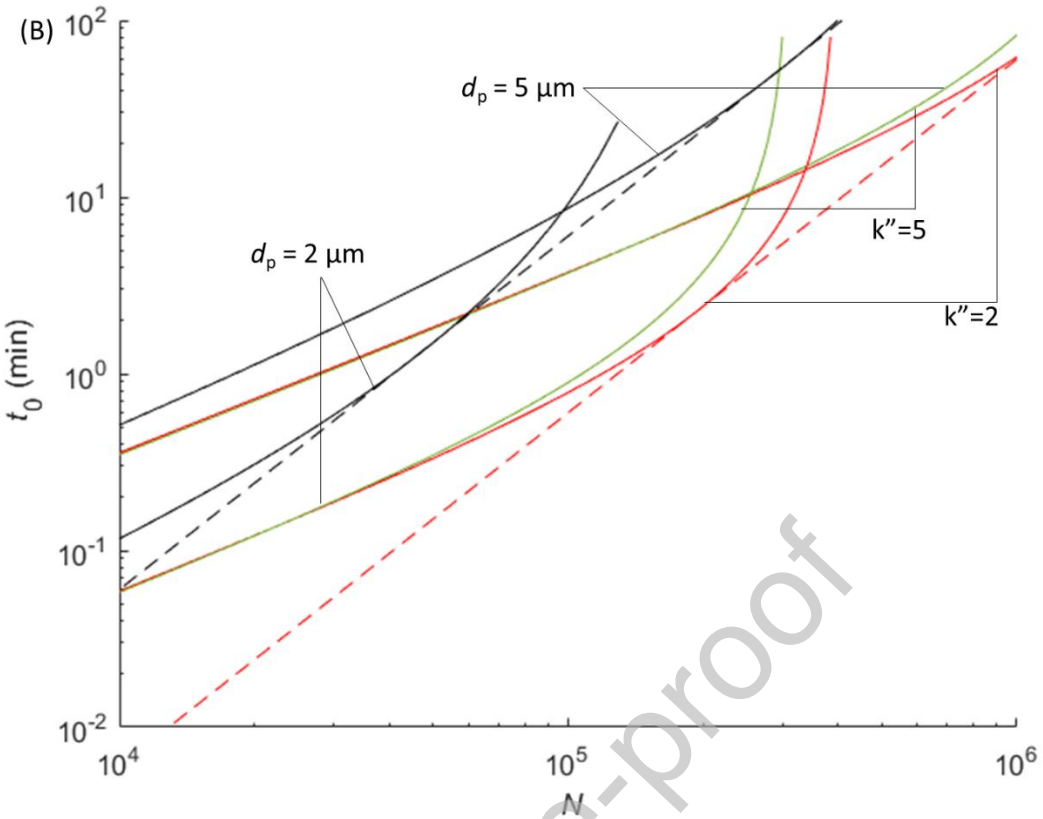
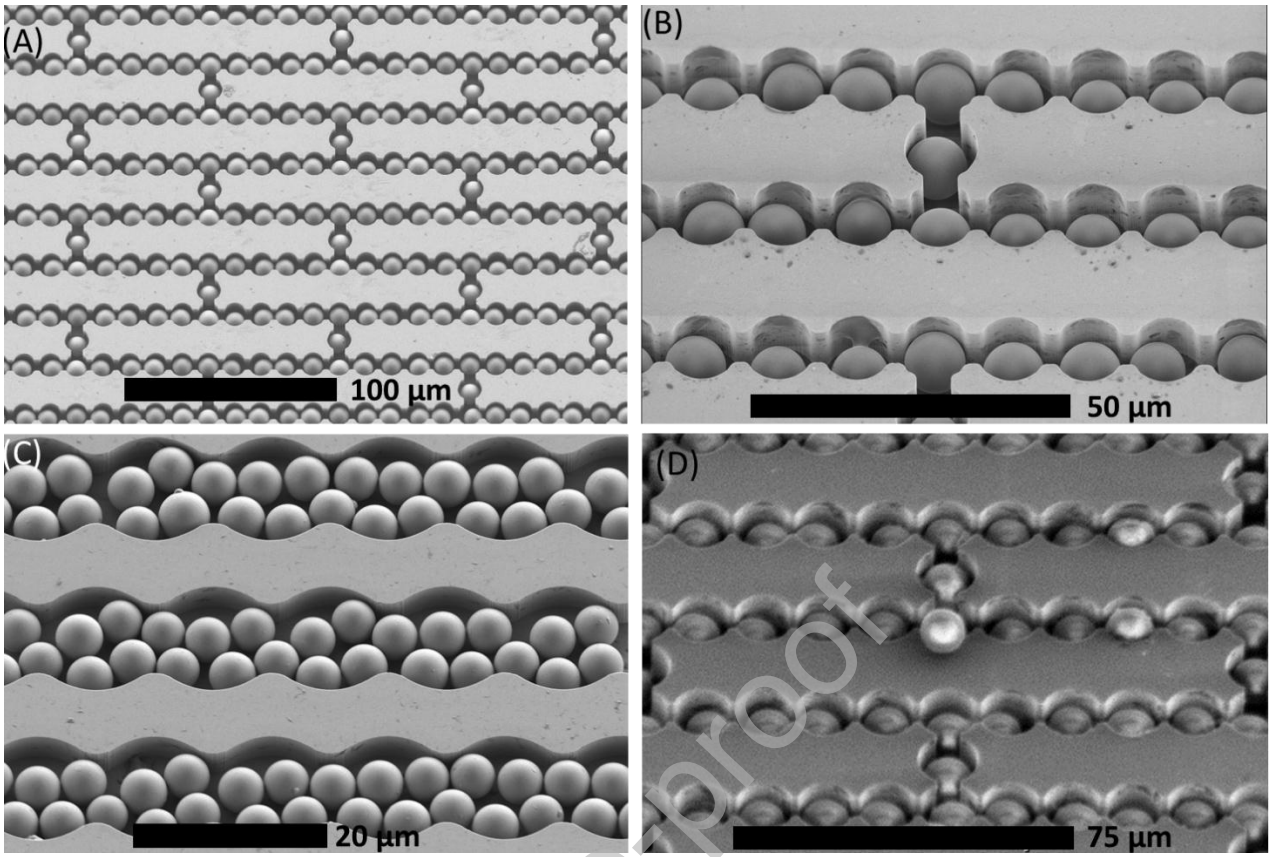


Figure 3. SEM images of micro-groove network columns filled with calibrated silica particles **(A, B)** micro-groove column filled with 10 µm silica particles, where each pocket is filled with exactly one particle. **(C)** micro-groove column filled with multiple particles per pocket. **(D)** SEM-image of an occasional problem (presence of excess particle sticking firmly to the silicon) preventing proper column sealing.



CReDiT Author Statement

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:
Gert Desmet reports a relationship with Thermo Fisher Scientific that includes: consulting or advisory.