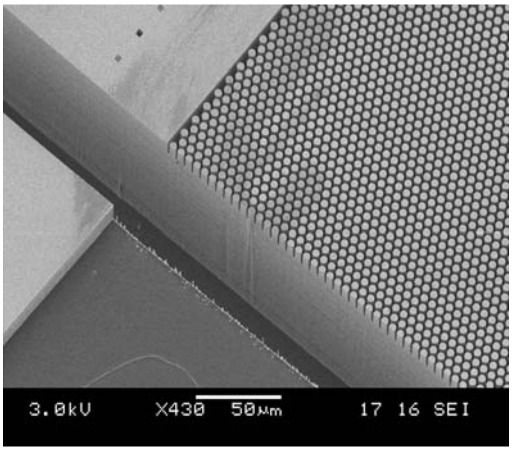
Abstract

The present thesis is situated in the field of high performance liquid chromatography (HPLC). Chromatography (in general) is probably the most used analytical technique, applied in a wide range of disciplines such as biology, chemistry, biochemistry, genetics etc. In most cases a particulate bed, packed with spherical micron-sized particles, is used as stationary phase. This work focused on improvements in high performance liquid chromatography (HPLC) by replacing the traditionally employed packed bed of spheres by micro-machined pillar arrays.

An array of micro-machined porous pillars would combine some of the advantages of a packed bed (high retention capacity and reproducibility of the column) with the advantages of a monolithic column (porosity can be tailored, low pressure drop), this while adding some of its own advantages (shape of the particles can be optimized, high degree of order).



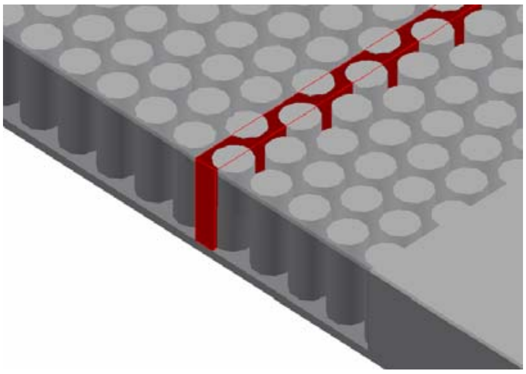


Figure: Overview of channel with etched pillars for HPLC separations and a computer-generated representation.

The idea to switch to micro-machined pillar array columns was originally proposed by Regnier *et al.* [1], but has not yet been brought into practice yet, partly because the optimization of the pillar design is insufficiently understood. In the present work, a commercial Computational Fluid Dynamics software package (fluent) has therefore been used to study the two main factors (pillar shape and the external porosity) determining the separation efficiency in micro-pillar array columns.

It was found that for fast separations pillar arrays with a small external porosity should best be used. Difficult separations requiring large plates numbers on the other hand are best pursued using high porosity arrays. The shape of the pillars has a smaller and a much more straightforward influence on the separation efficiency. It was found that the theoretical chromatographic performance limit can be most closely approached by using elongated pillar shapes with a large length/width ratio. In particular, diamond shaped pillars with large aspect ratio (1.7 or up, depending on the etching limitations) yield the best results if arranging the pillars according to the theoretically most advantageous positioning grid, i.e., one with a equilateral triangular pattern.

It was also investigated how the presence of the top and bottom cover plates leads to a significant additional source of band-broadening as compared to the band broadening that would prevail in a 2-D array. An equation allowing to estimate this additional plate height contribution has been established. This equation depends mainly on the etching depth and the width/depth of the pores and is independent on the internal porosity of the pillars or retention factor of the column.

Also making an extensive study of the effective diffusion, it was found that the obtained values deviated significantly from the traditionally employed expression for the B-term contribution, based on a time-weighted average of the mobile phase and stationary phase diffusion. The present study revealed that this assumption is invalid and that the effective diffusivity in a cylinder array should be regarded as being a mix of parallel and a series connection events of diffusion resistances. Based on this new insight, it was possible to establish a new and much more accurate procedure for the estimation of the stationary phase diffusion coefficient than possible with the time-weighted average expression.

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[1] He, B.; Tait, N.; Regnier, F.E. *Anal. Chem.* **1998**, 70, 3790-.