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Design and evaluation of microfluidic devices for two-dimensional spatial separations

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ABSTRACT

Various designs of chips for comprehensive two-dimensional spatial liquid chromatography were investigated. The performance of these chips was initially evaluated using computational fluid dynamics (CFD). A bifurcating distributor with an angle of 140° between branches was implemented in order to achieve a homogeneous velocity field. The cross-sectional area of the channels of the flow distributor was fixed at 0.5 × 0.5 mm, which allows a robust micromilling technique to be used for chip manufacturing. Experiments were performed with chips featuring purposely introduced imperfections in the structure of the bifurcating flow distributor to study its capacity of overcoming potential local clogging. Split peaks were observed when 75% of one of the flow channels was obstructed, in line with the CFD predictions. The main bottlenecks for the performance of the spatial two-dimensional chips were identified, *viz.* sample injected in the first dimension diverging into the flow distributor and channel discretization (*i.e.*, remixing of first-dimension separation peaks because of finite number of second-dimension channels). Solutions to the former problem were studied by applying a flow resistance in the vertical segments that formed the outlets of the flow distributor and by simulating the presence of constrictions. It was found that a flow resistance of 1.0×10^{11} m^{-2} reduced the amount of sample diverging into the flow distributor by a factor of 10. The presence of a constriction of 90% of the segment area and 50% of the segment length decreased the diverging flow by a factor of 5. The influence of the linear velocity was significant. Solutions to the channel discretization problem were sought by investigating different designs of spatial two-dimensional chips.

1. Introduction

Two-dimensional liquid chromatography has been receiving a great deal of attention in recent years [1,2]. One important reason for the interest in comprehensive two-dimensional liquid chromatography (LC × LC) is the high separation power of the technique, which is needed for the analysis of complex samples. Two main operating modes can be distinguished, *i.e.*, separation in time (^{t}LC), where different compounds are eluted at different times and separation in space (^{x}LC), where different compounds end up located in different positions in the separation body. Column-based chromatography is the prime example of the former; thin-layer chromatography is an example of the latter. Two-dimensional column-based (time-based) liquid chromatography (^{t}LC × ^{t}LC) has gained interest for the separation of peptides, proteins, pharmaceuticals [3,4], oils [5] and aromatic compounds [6]. In polymer science LC × LC has already been established as an indispensable technique for the characterization of polymers featuring multiple distributions [7,8].

The total peak capacity that can be obtained in ^{t}LC × ^{t}LC (ideally the product of the peak capacities in the individual dimensions) may be reduced by "undersampling" of the first-dimension separation and by injection band broadening in the second dimension [9,10]. Spatial two-dimensional LC (^{x}LC × ^{x}LC) does – in principle – not suffer from these undesirable drawbacks. Another advantage of this technique is that the analysis time is limited to the times needed for the least retained compounds to progress to the end of the separation bed in the first (^{1}D) and the second (^{2}D) dimensions. All ^{2}D separations are conducted at the same time, rather than sequentially, as is the case in column-based ^{t}LC × ^{t}LC separations.

Implementing an ^{x}LC × ^{x}LC system in a miniaturized format yields additional benefits, such as a lower solvent consumption and

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less dilution. However, some crucial issues need to be resolved if we are to realize successful spatial two-dimensional separations. One of the conditions to make full use of the potential peak capacity of a two-dimensional system is to implement two fully orthogonal retention mechanisms. Polymeric monolith stationary phases are attractive from this perspective. Firstly, they can be prepared *in situ* and it is possible to control the position of the monolith and to use different channel geometries [11,12]. Secondly, it is possible to modify the chemistry of the monolith through (post-polymerization) grafting to create very different stationary phases [13,14]. In ^{x}LC × ^{x}LC the detection is not as straightforward as in the case of ^{t}LC × ^{t}LC. Optical methods or MS imaging may be used to perform detection in the two-dimensional plane. However, finding a suitable detection method remains a problem for ^{x}LC × ^{x}LC.

For efficient spatial ^{x}LC × ^{x}LC adequate flow control is essential. To perform identical ^{2}D separations in parallel this comes down to realizing a good flow distributor, which ensures an equal flow at any point along the ^{1}D axis. In our previous paper, we concluded that bifurcating distributors (where in every row the channel divides into two channels to form the following row), outperformed radially interconnected flow distributors [15]. When designing a spatial two-dimensional separation system, another crucial aspect is that the ^{1}D flow should be strictly contained to the ^{1}D separation channel. In Ref. [16] this was achieved by incorporating a physical barrier between the flow distributor and the ^{1}D separation channel. While greatly improving the occurrence of sideways leaking into the flow distributor, the barrier also leads to considerable tailing when emptying the ^{1}D channel into the ^{2}D space.

Typical technologies for prototyping microfluidic chips include photolithography [17,18], soft lithography [19], hot embossing [20] and micromilling [21]. The photolithographic process encompasses several steps, for example, coating a wafer with a photoresist layer, selective exposure to light, dry or wet etching, and removal of the photoresist layer. The minimum feature size for common photolithographic processes is around 1 μm, while for micromilling this value is much higher (at least 100 μm). However, micromilling is an attractive technique, because it allows rapid prototyping of different designs and because it does not require cleanroom facilities. All microfluidic LC systems must be closed, usually by bonding of an upper substrate.

In the present study different design aspects of spatial two-dimensional chips are investigated using computational fluid dynamics (CFD). Bifurcating distributors are implemented to achieve the best possible flow patterns for ^{2}D separations [15]. The results of the CFD calculations are experimentally validated by flow experiments performed on microfluidic chips manufactured using micromilling techniques. The main bottlenecks for such a system – leak flow into the flow distributor and channel discretization – are addressed.

2. Experimental

2.1. Chemicals and materials

Cyclohexane (anhydrous, 99.5%) was purchased from Sigma–Aldrich (Diegem, Belgium), 2-propanol (technical) was acquired from VWR (Leuven, Belgium), and Red-40 dye was purchased from Kroger (VA, USA). TOPAS (grade S8007-04) cyclic-olefin-copolymer substrate plates (10 × 10 × 2 mm) were purchased from Kunststoff-Zentrum (Leipzig, Germany). Nanoports were purchased from Upchurch Scientific (Oak Harbor, WA, USA).

2.2. Prototyping spatial LC × LC microfluidic chips

Microfluidic spatial ^{x}LC × ^{x}LC chips featuring different configurations of bifurcating flow distributors were designed using AutoCAD software (Autodesk, San Rafael, CA, USA). Machining jobs were created using PrimCAM (Primus Data, Einsiedeln, Switzerland). A Datron M7Compact (Datron, Mühltal-Traisa, Germany) numerically-controlled micromilling robot was used to face-mill the substrate plate (reducing the surface waviness down to 30 μm) and to machine the desired channel layouts. Milling conditions were optimized for the specific grade of COC used, such as to prevent melting of the substrate and to minimize the formation of burrs. The spindle speed was set at 17,500 rotations per min. For drill diameters ≥ 2 mm in diameter the cut-off depth and feed rate were set at 50 μm and 3000 mm/min, respectively. The cut-off depth and feed rate for drills with diameters ≤ 0.5 mm were set at 75 μm and 500 mm/min.

A solvent-vapour-assisted bonding approach was optimized to yield pressure-resistant microfluidic devices. Cover plates were exposed for 7 min to cyclohexane vapour by suspending them 5 mm above liquid cyclohexane held at 25 °C in a closed container. Next, the top substrate was aligned with the bottom substrate featuring the channel layout, and the two substrates were pressed together for 60 min by applying a force of 2.5 kN.

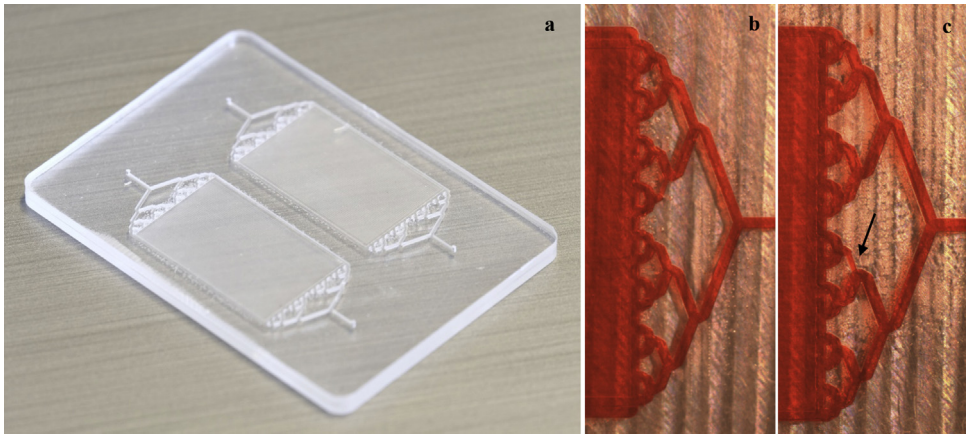

Fig. 1. (a) Photograph of a microfluidic chip. The channel layout is machined in the bottom plate and a sealing plate is irreversibly bonded on top. Each chip features two different designs, consisting of a bifurcating flow distributor with an angle of 140° between branches and a cross-sectional area of 0.5 × 0.5 mm, 16 outlets and a wide open channel with cross-sectional channel area of 19.2 × 0.5 mm. (b) Detail of the flow distributor without constrictions. The channels have been filled with a red dye for the sake of clarity. (c) Detail of a flow distributor with a constriction of 75% introduced in the third row of the bifurcating distributor (indicated by the arrow) by micromilling a shallower, narrower section in the channel.