Design and fabrication through additive manufacturing of devices for multidimensional LC based on computational insights

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Chapter 4

*Passive flow-confinement in devices for spatial multi-dimensional liquid chromatography*

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**This chapter relates to the following publication:**

Abstract

In spatial multi-dimensional liquid chromatography (LC) devices the flow of each dimension has to remain in the corresponding region, otherwise the separation efficiency is undermined. Adequate flow-confinement measures are necessary. Here, the use of permeability differences across different compartments of spatial two-dimensional (2D) and three-dimensional (3D) LC devices as a method to guide fluid flow and reduce analyte loss during the first, second- and third-dimension development was investigated with computational fluid dynamics (CFD) simulations. In case of 2DLC devices, it was shown that porous barriers with a permeability on the order of $10^{-12}$ m$^2$ suffice to keep the total sample spillage from an open 1D channel under 1%. In case of 3DLC devices, it was shown that flow confinement could be achieved using an open 1D channel in combination with a highly-permeable monolith (permeability on the order of $10^{-12}$ m$^2$) in the second-dimension (2D) and a less permeable packing with a permeability on the order of $10^{-15}$ m$^2$ (e.g. 1 μm particles) in the third-dimension (3D). Additionally, the impact of the 3D flow-distributor has been studied and a novel design, capable of limiting the spillage to the other dimensions to the absolute minimum, is proposed.
Chapter 4

4.1. Introduction

Comprehensive two-dimensional liquid chromatography (LC×LC) has emerged in the last two decades for separating complex samples that require more separation power than can be provided by one-dimensional liquid-chromatography systems [1,2]. Column-based, “temporal” multi-dimensional systems (such as LC×LC) help address the need, but they suffer from long analysis times. A solution can be provided by spatial two- and three-dimensional systems, which offer higher efficiencies along with relatively short analysis times. To conduct spatial multi-dimensional separations (LC×LC and LC×LC×LC) devices with two or more interconnected sets of separation bodies need to be constructed [3]. First, separation will take place in the first-dimension (1D) channel. After the 1D separation, the analytes will be transferred from the 1D channel to the second-dimension (2D) region with the aid of a 2D flow-distributor and they will be separated further. In the case of LC×LC×LC, the analytes will be transferred from the second to the third dimension (3D) with a 3D flow-distributor, located on top of the 2D region and they will be separated even further.

Several aspects of such devices have been investigated over the last decade, including chip manufacturing [4], flow distributor designs [5,6] and stationary-phase implementation [7–9].

Flow confinement is necessary during all steps in the operation of such devices. Spillage to compartments other than the intentioned ones will lead to losses in performance. For the creation of a chip that combined iso-electric focusing (IEF) with reversed-phase (RP) LC (i.e. IEF×RPLC) for the separation of peptides, Liu et al. used geometrical constrictions with 700 μm length, 85 μm width and 60 μm depth to achieve flow confinement between dimensions. Successful confinement was demonstrated during the IEF separation [10]. Wouters et al. used an offset alignment of micro-milled chip layers to create through-holes with ten times smaller cross-sectional area than the 1D channel to limit the spillage [3]. While effective in achieving flow confinement, the fabrication method significantly limits the geometrical conformity of possible chip designs and layouts. It also limits the manufacturing
method to the bonding of separate layers of chips, implying that the versatility in geometries and materials provided by other techniques, for example 3D-printing [11], cannot be utilized.

In the present study, differences in permeability and novel flow-distributor designs were studied as flow-confinement strategies. Computational-fluid-dynamics (CFD) simulations were used to establish the effectiveness of various strategies. In spatial 2DLC devices, the efficacy of porous barriers to prevent spillage of the 1D mobile phase and analytes into the flow distributor and the 2D separation zone was investigated. The added flow-resistance of the porous barriers can serve to guide fluid-flow during the 1D step of the separation. A practical solution would be the use of polymer monoliths, which are often used as frits to contain packed stationary phases [12–14]. The 1D injection step is studied for several permeability values of the flow-distributor barriers ($K_{f db}$) and 2D separation zone ($K_2$). Concerning spatial 3DLC devices, the flow-confinement effectiveness of permeability differences between 2D ($K_2$) and 3D ($K_3$) regions were studied, as well as the impact of the third-dimension flow-distributor design.

4.2. Materials and Methods

4.2.1 Chip geometry and design

Microfluidic devices for spatial 2D- and 3DLC were designed using SOLIDWORKS (Dassault Systèmes SOLIDWORKS, Waltham, MA, USA) and Autodesk Inventor (Autodesk, San Rafael, CA, USA). In Fig. 4.1 four geometries are illustrated; the spatial 2DLC geometry is presented in A, the simplified geometry for spatial 3DLC in B. Figures 4.1C and 4.1D show a simplified geometry for spatial 3DLC, but with the addition of a 3D flow-distributor (see also Fig. 4.9 below). Furthermore, the 2DLC device in frame A comprises a 2D bifurcating flow-distributor with internal diameter of 300 μm, a 1D channel with 24 mm length and 1.5 mm width and height, a 2D flat bed with 24 mm length, 10 mm width and 1.5 mm height and porous barriers at the outlets of the flow-distributor (indicated in black). In frame B, the geometry consists of a 1D channel with 24 mm length and 1.5 mm width and height, a 2D flat bed with
24 mm length and width and 1.5 mm height and a 3D cube of 24 mm length and width and 20 mm height. The 2D and 3D flow-distributor were omitted in order to reduce the computational cost of the simulations. In frame C, the 3D flow-distributor added to the setup is a combination of an H-shaped distributor (starting from 1 to 16 splits) on the top and then bifurcating vertically to the bottom resulting to 256 outlets (type A). Finally, in frame D a novel design for flow-distribution prior to the 3D separation is presented. It consists of 2D flow-distributors stacked along the direction of the flow of the 2D development, and then connected at the top with another 2D flow-distributor. Both distributors contain channels with a diameter of 700 μm. The novel design – referred to as type B – was proposed to avoid recirculation and mixing during the 2D injection on a spatial 3DLC device. The requirements for manufacturing these devices using 3D-printing techniques are well within the current state-of-the-art. All dimensions were chosen with respect to achievable printing resolutions.
Fig. 4.1. The four geometries used in this study (isomeric view of the designs); (A) the spatial 2DLC geometry, (B) the simplified geometry for spatial 3DLC, (C) a simplified geometry for spatial 3DLC with the addition of a type-A flow distributor, (D) as (C), except with a type-B 3D flow distributor. \( K_{\text{fdb}} \) indicates the permeability of the 2D flow-distributor barriers, \( K_2 \) the 2D bed permeability, \( K_3 \) is the 3D Cube permeability and \( K_{3DFD} \) is the permeability of the imposed porous zone in the top part of the 3D flow distributor (see also Fig. 4.9).

4.2.2 Computational fluid dynamics simulations

For all CFD simulations, ANSYS Workbench Fluids and Structures Academic package (versions 16.2-17.2) was used (ANSYS, Canonsburg, PA, USA). All types of devices studied were discretized in a similar manner with ANSYS Meshing.
Smaller sized cells were used in the regions of interest, to increase the accuracy of the computations.

In cases in which devices for spatial 2DLC were examined, the 1D channel was meshed with a fixed element size of 70 μm, while the 2D flat bed was meshed with a structured grid of hexahedral cells, resulting in 288 cell layers in the direction of 1D development and 30 cell layers in the direction of 2D development. The 2D flow-distributor was meshed with tetrahedral cells in combination with inflation layers of wedge-shaped cells near the distributor walls. Tetrahedral meshing was chosen in order to obtain a higher quality mesh as well as for the compatibility with inflation which was necessary at the flow-distributor. The total number of elements in this computational setup was around 2 500 000. Regarding the spatial 3DLC geometry setups, three geometries were examined, i.e. one to study the flow-confinement between the 2D and 3D developments and two to examine the effectiveness of the 3D flow-distributor during the 2D injection. For the first study, the geometry comprised only a 1D channel, a 2D flat bed and a 3D cube (Fig 4.1B). This geometry was meshed with a fixed element size of 15 μm on the 1D channel, 200 μm in the 2D and 500 μm in the 3D. In this study only hexahedral cells were used. For the other two studies, the examined devices comprised all previously referred compartments, with the addition of the 3D flow-distributor (see Figures 4.1 C and 4.1D). In these cases, meshing was done in a similar manner, except that we used tetrahedral cells in the 2D region and in the 3D flow-distributor and that we incorporated inflation on the latter. In both cases the number of elements was around 8 500 000. For all cases included in this work, the solution was independent of the grid size.

All simulations were conducted using the Fluent solver and they were solved for flow [15] and species-transport. In the latter case, Fick’s law for diffusion applies [16]. In Table 4.1 all the examined cases are summarized, alongside the permeability values used.
4.2.3 Flow confinement in spatial 2DLC setup

In order to achieve a sufficiently high permeability difference between the 1D channel and its surroundings, such as to avoid spillage either to the distributor or to the 2D region, the 1D channel was empty (i.e. no packed bed or monolith present). This concurs with previous 2D cases studied in this thesis (see Chapter 3). To achieve separation in the empty 1D channel, iso-electric focusing (IEF) may feasibly be used as the first-dimension separation mechanism. Although there is no pressure-driven flow in IEF during the separation and, thus, there is no pressure gradient along the channel, spillage may still occur during filling of the 1D channel. The 2D region was a flat bed for the sake of simplicity. Porous zones were present in the outlets of the flow-distributor (see Fig. 4.1A, zone indicated in black). Their performance as barriers was investigated through parameterization of imposed permeability values of these porous zones ($K_{f\text{db}}$) (see Table 4.1, cases I-VI), while the second dimension had a constant permeability value, simulating the presence of a stationary phase with $K_2 = 1.17 \times 10^{-13}$ m$^2$. Additionally, the effect of the permeability of the 2D flat-bed was studied by keeping the permeability of the porous barriers constant at $K_{f\text{db}} = 10^{-12}$ m$^2$ and varying the permeability of the 2D region (see Table 4.1, cases VII-XI). To investigate possible spillage, a mixture of dye and water was injected at 5 mL per minute with an injection volume corresponding to three 1D channel volumes. During the 1D injection the 2D inlet and outlet remained closed. The spillage at the 2D flow-distributor and the 2D was quantified by using Eq. 4.1 and 4.2

$$Spillage\%_{\text{2DFD}} = \frac{\int \phi_{\text{2DFDB}} \rho dV}{\int \phi_{\text{total}} \rho dV} \times 100 \quad (4.1)$$

$$Spillage\%_{\text{2D}} = \frac{\int \phi_{\text{2D}} \rho dV}{\int \phi_{\text{total}} \rho dV} \times 100 \quad (4.2)$$

where $\int \phi_{\text{2DFDB}} \rho dV$ is the dye mass-weighted integral (kg/m$^3$)(m$^3$) in the 2D flow-distributor, $\int \phi_{\text{2D}} \rho dV$ in the second-dimension region and $\int \phi_{\text{total}} \rho dV$ is the total dye mass-weighted integral present in all compartments of the device, where $\phi$ is the mass fraction of dye, $\rho$ is density and $V$ is the cell volume.
Table 4.1. Permeability values of the 2D flow-distributor barriers, the 2D flat bed for the spatial 2DLC cases, the 3D cube for the spatial 3DLC cases, the top part of the 3D flow-distributor (FD) and an indication of the type of the 3D FD. Parameters that are non-applicable are indicated by grey boxes.

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<th>Case identifier</th>
<th>2D Flow Distributor Barriers permeability ($K_{fd}$) [m$^2$]</th>
<th>2D Bed permeability ($K_2$) [m$^2$]</th>
<th>3D Cube permeability ($K_3$) [m$^2$]</th>
<th>3D FD (top part) permeability ($K_{3FD}$) [m$^2$]</th>
<th>3D FD type</th>
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<td>type B</td>
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</table>
4.2.4 Flow confinement in spatial 3DLC setup

4.2.4.1 Permeability values for efficient flow confinement between 2D and 3D regions

During the 2D operation of a spatial 3DLC separation, the flow and the analytes have to be confined to the compartment of the device corresponding to the second dimension. Any spillage to the third dimension can undermine the overall performance of the device. In order to study the control of the flow, different permeability values were used in the 2D \( (K_2) \) and 3D \( (K_3) \) regions. The geometry used here is depicted in Fig. 4.1B and the examined combinations of permeability values are listed in Table 4.1. Both realistic and unrealistic combinations were studied. Realistic cases (see Table 1, cases XII-XV and XX-XXI) were investigated in order to define the minimum percentage of dye spillage to the 3D region that can be achieved. Cases that exceeded the current state of the art in terms of achievable permeabilities (see Table 4.1, cases XVII-XIX) were included to study in more detail the percentage of spillage percentage caused by diffusion between the 2D and 3D regions, defined as:

\[
Spillage\%_{3D} = \frac{\int \phi \, z_3 \rho \, dV}{\int \phi_{total} \rho \, dV} \times 100
\]  

(4.3)

The initial condition of the simulations was a perfectly filled 1D channel with a mixture of dye and water (dye 1%), which was flushed during the 2D injection to the second dimension flat bed at an average velocity of 1mm/s. Equation (4.3) was used for calculating the percentage of dye spillage to the third dimension, with \( \int \phi \, z_3 \rho \, dV \) corresponding to the dye mass-weighted integral in the 3D region. During these simulations the 1D channel contained no stationary-phase material and the 2D inlet and outlet were open, while all other inlets and outlets were closed.
4.2.4.2 Impact of the 3D flow-distributor and novel design

The impact of the third-dimension flow-distributor on the flow profile was examined during the second- and third-dimension developments. During the second-dimension development, a continuous injection of three 2D volumes of a mixture of dye and water was performed to determine the spillage of dye to the 3D flow-distributor. In cases XXII and XXIII (see Table 1) the flow-distributors were empty, while in cases XXIV and XXV the upper section of the flow-distributors was set to be a porous zone in order to investigate the effectiveness of additional resistance for successful flow confinement to the second dimension. Eq. (4.4) was used for calculating the percentage of dye spillage to the 3D flow-distributor, with $\int \phi z_{DFD} \rho dV$ corresponding to the dye mass-weighted integral in the 3D flow-distributor.

$$Spillage\%_{3DFD} = \frac{\int \phi z_{DFD} \rho dV}{\int \phi_{total} \rho dV} \times 100 \quad (4.4)$$

After comparing the designs and operating conditions of the 3D flow distributors during the 2D development, the performance of the device was examined during the 3D development. The initial condition for this simulation was a completely filled 2D flat-bed with a mixture of dye and water (1% dye). Water was injected from the 3D inlet and the flow rate was adjusted in order to attain an average velocity of 1 mm/s in the 3D region. The 3D injection occurred for one 3D-region volume (11.424 mL).

4.3 Results and Discussion

4.3.1 Flow-confinement in spatial 2DLC setup

In this section, the case of a spatial 2DLC device consisting of a 2D flow-distributor with porous barriers close to the 1D channel, a 1D channel and a 2D flat-bed with porous media was examined. The goal of this study was to determine suitable permeability values for the porous barriers and for the 2D flat bed to achieve flow confinement.
4.3.1.1 Effect of flow-distributor barriers

To examine the performance of the barriers situated in the FD, different permeability values were examined while the value of the 2D flat-bed remained constant at $K_2 = 1.17 \times 10^{-13}$ m$^2$.

It is noticeable that all spillage values were below 0.5%, implying that with the permeability values tested here, less than 1% of the 1D mobile phase can be expected to spill out of its intended region. This indicates good flow control within the device when $K_2 = 1.17 \times 10^{-13}$ m$^2$, regardless of $K_{fdb}$. As seen in Fig. 4.2, the spillage into the distributor reduced drastically with decreasing barrier permeability until a value of $10^{-12}$ m$^2$ was reached. Lower $K_{fdb}$ values resulted in negligible improvements in flow control. Conversely, spillage into the 2D region remained nearly identical for all tested $K_{fdb}$ values at roughly 0.49%. Probably, this remaining contribution is due to the inevitable molecular diffusion (see also discussion of Eq. (4.5) below).

![Image of Fig. 4.2](image_url)

**Fig. 4.2.** Dye spillage to the 2D flow distributor (in black) and to the 2D flat-bed (in white) as a function of the permeability of the barriers in the flow distributor ($K_{fdb}$) with a constant permeability of the 2D flat bed ($K_2 = 1.17 \times 10^{-13}$ m$^2$).
**Fig. 4.3.** Contour plots of mass fraction of dye after 1D injection of three 1D channel volumes (zoomed-in top view of the geometries); (A) case III with $K_{fdb} = 10^{-11}$ m$^2$ and (B) case IV with $K_{fdb} = 10^{-12}$ m$^2$.

However, despite the seemingly good flow control suggested by Fig. 4.2, spill-over into the flow distributor remains an issue. Fig. 4.3A shows the dye present in the flow distributor to be unevenly distributed across the distributor outlets, even with barriers with a permeability of $10^{-11}$ m$^2$. In addition to contributing to band broadening during the 2D separation, the spill-over pattern seen in Fig. 3A can result in misplacement of analytes during the 1D step. A portion of an analyte that would otherwise be on the right of the 1D channel may be trapped within a flow distributor outlet on the left of the 1D channel during the 1D development. As seen in Fig. 4.3B, by reducing the permeability value by one order of magnitude, an even distribution of dye across the flow-distributor outlets can be achieved, while spillage is significantly reduced. However, flow-distributor barriers with lower permeability values ($K_{fdb}$) also cause additional back-pressure during the 2D separation, which is not desirable.
As a compromise, a permeability value of $K_{\text{fdb}} = 10^{-12} \text{ m}^2$ was selected as the most preferable. In practice, flow-distributor barriers with permeabilities of $K_{\text{fdb}} \geq 10^{-12} \text{ m}^2$ can be achieved by locally polymerizing monolithic frits [17].

### 4.3.1.2 Effects of 2D Bed Permeability

In this section, the impact of the permeability of the 2D flat bed was investigated, while the permeability of the porous barriers in the flow distributor remained constant. As demonstrated in Fig. 4.2, spillage into the 2D region is greater than spillage into the flow distributor for all tested $K_{\text{fdb}}$ values, indicating that the permeability of the 2D region ($K_2$) may play a larger role in controlling total spillage. Fig. 4.4 shows dye spillage into both regions as a function of $K_2$, with $K_{\text{fdb}} = 10^{-12} \text{ m}^2$. A drastic reduction in spillage into the 2D region was observed from $K_2 = 10^{-9} \text{ m}^2$ to $10^{-11} \text{ m}^2$. However, further reductions in permeability produced negligible improvements in flow-control. This fraction is the result of spillage due to pure diffusion (see also discussion of Eq. (4.5) below) and is, hence, insensitive to further decrease the bed permeability. The surface available for diffusion from the 1D to 2D is larger than the surface from the 1D to the flow distributor, explaining the higher spillage to the 2D. The dye-spillage percentages to the 2D flow distributor did not change significantly (ranging from 0.042 to 0.049%).
Fig. 4.4. Dye spillage to the \(^2\)D flow-distributor (in black) and to the \(^2\)D flat bed (in white, values on secondary y-axis) as a function of \(^2\)D bed permeability \(K_2\). Barriers in \(^2\)D flow distributor with constant permeability \((K_{fdb} = 10^{-12} \text{ m}^2)\).

Additionally, as can be seen in Fig. 4.5, the dye-spillage profile in the \(^2\)D became more uniform when reducing the permeability of the \(^2\)D domain. This effect could be achieved even with high permeability values in the order of \(K_2=10^{-11} \text{ m}^2\).
Fig. 4.5. Contour plots of mass fraction of dye after $^1$D injection of three $^1$D channel volumes (top view of the geometries) with $K_{fdb}=10^{-12}$ m$^2$. (A) case VII ($K_2=10^{-9}$ m$^2$) and (B) case IX ($K_2=10^{-11}$ m$^2$).

To summarize the design of spatial 2DLC devices, to be used, for example, for IEF×LC separations, a flow distributor with (monolithic) barriers ($K_{fdb}=10^{-12}$ m$^2$) and a 2D regions with a permeability of $K_2=10^{-12}$ m$^2$ can be effectively used to achieve passive flow-control within a device.

4.3.2 Flow-confinement in spatial 3DLC setup

The concept of spatial 3DLC consists of three consecutive developments, the first of which occurring in a $^1$D channel, the second in a $^2$D area which can be in the form of a flat-bed or discrete channels and the third in the form of a cube or discrete channels located perpendicularly to the $^2$D compartment. For the successful operation of such a device, each development must be independent of the other two. Consequently, the flow in each dimension has to be confined in the respective compartment of the device. Here, the permeability difference between the $^2$D and $^3$D regions was investigated as a flow-confinement mechanism during the $^2$D injection, as well as the effects of the shape and operating conditions of the $^3$D flow distributor during the second- and third-dimension developments.
4.3.2.1 Efficient permeability values for flow confinement between $^2$D and $^3$D regions

For the purposes of this study, the geometry shown in Fig. 4.1B was used. The computational setup consisted of a $^1$D channel without stationary-phase material, a porous $^2$D flat bed and a porous $^3$D cube. The $^2$D and $^3$D flow distributors were omitted here. Different permeability values were examined for the $^2$D and $^3$D regions (see Table 4.1; cases XII-XXI).

Fig. 4.6. Dye spillage to the $^3$D region as a function of $^3$D-cube permeability $K_3$; realistic cases are in white, borderline cases in grey and unrealistic cases (sub-micron particles) in black. Constant permeability of the $^2$D flat bed ($K_2=10^{-12}$ m$^2$).

Fig. 4.6 shows the dye spillage percentage into the $^3$D space during the $^2$D injection for different $^3$D permeabilities, while the permeability of the second dimension was kept constant ($K_2=10^{-12}$ m$^2$). The tested $K_3$ values ranged from realistically achievable ($K_3=10^{-13}$ m$^2$, corresponding with a packed bed with approximately 10-µm particles or a monolith with random globule chains [18]) to unrealistic ($K_3=10^{-25}$ m$^2$, corresponding with a packed bed with approximately 10-nm particles). With three orders of magnitude difference between the permeability of the $^2$D and $^3$D regions the
dye spillage is around 7% for a $K_3=10^{-15}$ m² (resembling packed bed with 1-µm particles). More information regarding permeability values and particle diameters is included in section 4.5.

Regarding the unrealistic cases ($K_3 \leq 10^{-16}$ m² or $\leq 0.3$ µm particles), the spillage was reduced to less than 7% and it remained constant for differences in permeability values greater than eight orders of magnitude, which suggested that this remaining percentage is caused by molecular diffusion into the 3D cube, rather than by convection. If this is the case, no further improvements can be anticipated by confining the flow to the 2D region.

To verify the molecular-diffusion hypothesis we can use the well-established solution for diffusion from a rectangular box [19].

$$C = \frac{C_0}{2} \left[ \text{erf}\left(\frac{w-x}{2\sqrt{D.t}}\right) + \text{erf}\left(\frac{w+x}{2\sqrt{D.t}}\right) \right]$$  \hspace{1cm} (4.5)

In this equation, $w$ is the width of the 2D region, $D$ is the diffusion coefficient, and $x$ is the spatial in the direction of 3D development ($x=0$ at the top of the bed, and $x=w$ at the end of the 2D region, see Fig. 4.7). By integrating the normalized concentration from $x=w$ to $x=+\infty$ we obtain the amount of species that has diffused out of the 2D “box”.

It is not straightforward to determine the time $t$ for which the diffusion process needs to be evaluated (the section near the inlet of the 2D region has been in contact with the species longer than the section near the outlet of the 2D region). Taking $t$ as the total time it takes to fill the entire 2D region (in the present case about 35 s), the equations predict a value of 6.95%, extremely close to the numerically observed value. It would, however, seem more appropriate to take the average time the 2D bed has been in contact with species (i.e. $(35+10)/2=22.5$ s, given that it takes 25 s for the species to flow from the inlet to the outlet of the 2D bed). Taking this value, we obtain a value of 5.56%, which is less than the observed 7%, but still in the right order. The slight underestimation may be attributed to the fact that the numerical calculations do take into account that the box is continuously fed with new tracer, while the calculation based on Eq. 4.5 assumes that no further species are supplied to the box after $t=0$. 

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Fig. 4.7. Contour plots of mass fraction of dye after the 2D flat-bed (red zone; side view of the geometry) was completely filled with dye ($K_2 = 10^{-12}$ m$^2$ and $K_3 = 10^{-15}$ m$^2$). Arrow indicates direction of 3D development (x direction).

In Fig. 4.8, the dye profiles are presented following 3D injection (i.e. transfer of the dye from a fully filled 1D channel) with one 2D volume of water. In Fig. 4.8A, permeability values were the same in the 2D and 3D regions, which led to the dye leaking to the third dimension. In Fig. 4.8B, three orders of magnitude difference between $K_2$ and $K_3$ were imposed, which led to the successful transfer of the dye from the first to the second dimension and the flow being confined to the 2D space.

Fig. 4.8. Contour plots of mass fraction of dye after 2D injection of one 2D flat-bed volume (side view of the geometry); A, case XII ($K_2 = 10^{-12}$ m$^2$ and $K_3 = 10^{-12}$ m$^2$); B, case XV ($K_2 = 10^{-12}$ m$^2$ and $K_3 = 10^{-15}$ m$^2$).
Furthermore, two additional permeability combinations were examined (see Table 4.1; cases XX & XXI), with $K_2 = 10^{-13}$ m$^2$ and $K_3 = 10^{-14}$ m$^2$ and $10^{-15}$ m$^2$. The spillage for case XX was 43.02%, which is similar to the 40.51% computed for case XIII. For both these cases there was one order of magnitude difference between the permeabilities ($K_2=10K_3$). For case XXI we found a spillage of 13.42%, which was identical to the spillage found for of case XIV. For both these cases the permeabilities differed by two orders of magnitude difference ($K_2=100K_3$).

This suggests that the ratio of permeability values between the 2D and 3D regions is more important than the absolute permeabilities for achieving flow-control. In practice, the difference of three orders of magnitude recommended here can be achieved using a highly-permeable monolith in the 2D region and a packed bed of 1-μm particles in the 3D region.

4.3.2.2 Impact of the 3D flow-distributor to flow-path and novel design

Two third-dimension (3D) flow-distributors (FDs) were examined for their performance during the second-dimension injection. One (Fig. 4.9A) was a combination of an H-shaped FD at the top part, connected to bifurcations ending up in 256 outlets and the other FD (Fig. 4.9B), also ending up in 256 outlets, with sixteen repetitions of the previously used 2D flow-distributor (see Fig. 4.1A) connected at the top with another 2D flow-distributor. In both cases, the spacing and internal diameter were identical, but the total internal volumes of the two FDs were different.
Fig. 4.9. The two types of 3D flow distributors used in this study (isomeric view of the designs); (A) type A; H-shaped part located at the top (in black), followed by the bifurcating part (in grey), (B) type B FD; sixteen repetitions of the 2D flow-distributor (in grey), connected at the top by another 2D flow-distributor (in black).

Fig. 4.10A shows the result in case of an “open” type A 3D flow-distributor (i.e. with empty flow channels). It can be seen that the flow diverged from the second-dimension area and followed the path of the least resistance, which led to an excessive dye spillage of approximately 40% (Fig. 4.11, case XXII). In Fig. 4.10B, the upper layer (region indicated in black in Fig. 4.9A) of the flow-distributor had a permeability of $10^{-13} \text{ m}^2$, which could be achieved by creating a monolith. The positioning of that zone was chosen because it was anticipated to be experimentally feasible, unlike the creation of a monolith in the entire third-dimension flow distributor. As shown in Fig. 4.10B, this leads to little improvement in flow control within the type-A 3D flow-distributor (Fig. 4.11, case XXIV).

When a type B distributor with empty channels was used (Fig. 4.10C) the flow also diverged from the 2D through the flow-distributor towards the 2D outlet and excessive spillage (47%, see Fig. 4.11, case XXIII) was observed (see Fig. 4.10C). However, when the top part (region indicated in black in Fig. 4.9B) contained a porous zone with a permeability of $10^{-13} \text{ m}^2$, the dye remained in the second-dimension flat bed, with a spillage of 5% to the 3D flow-distributor (Fig. 4.11, case XXV). From Eq. (4.5) it can be inferred that an important fraction of this 5% is likely due to molecular diffusion, which inevitably occurs, even in the absence of a convective leakage flow.
Fig. 4.10. Contour plots of mass fraction of dye during a continuous $^2$D injection. Results shown after the injection of three $^2$D flat-bed volumes (isomeric view of the geometries). (A) case XXII with the empty type A FD, (B) case XXIV with the type A FD and a porous zone imposed in the top segments, (C) case XXIII with empty type B FD and (D) case XXV with type B FD and a porous zone imposed in the top segments.
After the desired design and operating conditions of the 3D flow distributor during the 2D injection were determined, a 3D dye injection was simulated. The initial condition here was a perfectly-confined 2D region, fully-filled with dye. As presented in Fig. 4.12, the dye was successfully transferred from the second to the third dimension.

**Fig. 4.11.** Dye spillage to the 3D flow-distributor for the four cases illustrated in Fig. 4.10.

**Fig. 4.12.** Contour plots of mass fraction of dye after the 3D injection of one device volume; case XXV with $K_2 = 10^{-12}$ m$^2$, $K_3 = 10^{-15}$ m$^2$ and $K_{fdb} = 10^{-13}$ m$^2$ (isomeric view of the geometry).
A shortcoming that is apparent from Fig. 4.12 is the severe tailing at the wall located below the 1D channel. Clearly, a low concentration of dye is still present along that wall, as well as inside the 1D channel. The latter effect was caused by the absence of a barrier between the first and the second dimension. Starting the 3D development with a completely filled 2D flat bed and an empty 1D channel, some dye first entered the 1D channel, which contains a stagnant liquid during the 3D development. The dye was then washed out very slowly towards the 3D cube. This dye spillage amounted to 0.02%, which, while low, could still be troublesome in case of actual 3D separations. To prevent the above effects a barrier can be inserted between the first and the second dimension. Promising options are a rotating valving mechanism, such as the TWIST (see Chapter 3), or a freezing and thawing valve system used in the COSMIC device [20]. In the first case, the device would be modular in the first dimension (two-dimensional insertable separation tool, hence TWIST). In the second case it would be a single-piece device with additional external jackets surrounding the 1D channel to allow flow of heating and cooling liquids to realize the valving mechanism. In the aforementioned cases, the 1D channel could also contain stationary-phase material.

### 4.4. Conclusions

Flow control and confinement are necessary for the successful operation of devices for spatial 2D and 3DLC. From this perspective, differences in permeability and design features were investigated in this study. In case of devices for spatial two-dimensional liquid chromatography with an open first-dimension (1D) channel (i.e. without a stationary-phase material), our study showed that porous barriers at the outlets of the second-dimension (2D) flow distributor can be used to prevent spillage to the flow distributor while injecting at the 1D inlet. The necessary permeability can be obtained by creating monoliths through selective local polymerization [17,21]. Highly permeable monoliths suffice to prevent spillage to the 2D compartment. The difference in permeability values between the second and the third dimensions was found to affect the flow confinement in devices for spatial three-dimensional liquid chromatography. The minimum spillage observed could be explained by
molecular diffusion, which represents an unyielding lower limit for the spillage. The simulations suggest that the ratio of permeabilities between dimensions plays a crucial role in flow control. Finally, the impact of the third-dimension flow distributor (FD) was studied. Two flow distributors were examined, viz. a type A or H-shaped distributor with bifurcations and a type B distributor consisting of sixteen 2D bifurcating distributors connected with another 2D flow distributor at the top. The latter was proposed to prevent recirculation and mixing during the 2D development. It was shown that the two 3D flow distributors performed similarly when the channels were open (i.e. containing no monoliths or particulate packing), giving rise to a dye spillage of around 40%. When the top part of both flow-distributors was set to contain a porous zone, excessive dye spillage still occurred in the device with the type A FD, while the dye spillage was reduced significantly for the device with the type B FD. Moreover, during the 3D development the vast majority of the dye was transferred from the 2D to the 3D. However, a small percentage entered the 1D channel before being inefficiently transferring to the 3D, which caused tailing on the one side of the 3D cube. For this reason, it would be advisable to use flow-confinement methods based on physical barriers between the 1D channel and the 2D region [16,20]. The operation of the device can still be successful, even without physical barriers between the 1D and the 2D regions, but it is anticipated that the total separation power of the system will be significantly reduced.

To summarize, two non-modular devices for spatial multi-dimensional liquid chromatography were proposed. For 2DLC a device consisting of a bifurcating 2D FD with monolithic frits close to the outlets, an open 1D channel and a 2D region filled with monolith or particles was presented. The proposed 3DLC device consisted of a bifurcating 2D FD with monolithic frits close to the outlets, an open 1D channel, a highly permeable monolith in the 2D region, a packed bed consisting of 1-μm particles in the 3D region and a type B 2D FD with additional resistance in the upper sections. A major advantage of the passive flow-control measures suggested here is that no additional equipment or processes will be necessary to achieve an effective spatial 2DLC or 3DLC separation. For the successful fabrication and operation of these
devices, a number of challenges will have to be addressed, including the precise formation of monoliths in the 2D channels and homogeneously packing the 3D channels with particles.

Finally, computational-fluid dynamics-simulations were proven to be valuable for the purposes of this study. The quantification of spillage would not have been possible under experimental conditions. As a result of this study, device designs and stationary-phase materials were proposed for the construction of successful devices for spatial multi-dimensional liquid chromatography.

4.5 Supplementary information

For the calculation of the permeability of the open 1D channel, Darcy’s law was used. The calculated value was $1.47 \times 10^{-7}$ m$^2$.

In order to translate the permeability values to monolith domain size in the 2D, cylindrical TSM structure was assumed with external porosity of 0.3. For cylindrical TSM the relations

$$
\frac{d_s^2}{K} = 51 \left( \frac{1-\varepsilon_e}{\varepsilon_e} \right)^{1.59} \quad \text{and} \quad d_{\text{dom}} = d_{\text{por}} + d_s
$$

are applicable [18]. With $d_{\text{por}}$ assumed 2.23, $d_{\text{dom}}$ is 16.23µm for a permeability value of $10^{-12}$ m$^2$ and 7.02µm for $1.17 \times 10^{-13}$ m$^2$.

The used permeability values were translated to particle size by solving the Kozeny-Carman equation. The Kozeny-Carman constant was assumed 180 for packed beds [22] and we used an external porosity ($\varepsilon_e$) of 0.4 assuming medium-porosity particles [23]. The obtained values are presented in Table S4.1.

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<th>$K$ [m$^2$]</th>
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References


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