Purification of proteins and nanoparticles by continuous field-flow fractionation

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6 Discussion and future perspectives
Two-dimensional field-flow fractionation (2D-FFF)

In the previous chapter, a novel 2D-AF4 system was presented in which microstructured membranes, having slanted grooves on their surface, allowed the continuous size-based fractionation of the sample components. However, in general the idea of 2D-FFF is by no means novel since the introduction of such systems began in 1979, when Myers and Giddings [1] presented a continuous steric FFF device for the size-based fractionation of microparticles. Two-dimensional field-flow fractionation [2] is referring to the coupling of an FFF subtechnique with another displacement process (that belongs or not to the FFF family) where the solutes exhibit different selectivity. These two processes may occur sequentially (configured off-line, online or in the same planar structure) or simultaneously at right angles (to achieve continuous fractionation). Continuous systems offer higher throughput, less dilution and it is easier to operate and integrate them with other continuous platforms. These characteristics make them suitable either for preparative purification or for microfluidic systems.

Off-line and online coupling of AF4 has been reported with liquid chromatography [3] and capillary electrophoresis [4], and a custom-made multilane 2D-AF4 system in which isoelectric focusing was followed by AF4 in the same planar geometry [5]. Few continuous FFF systems have been developed [1, 6] and continuous AF4 systems have not been previously reported. Nevertheless, considering the numerous possibilities of two-dimensional field-flow fractionation [2, 7, 8], the field seems unexploited. It is anticipated that due to the increasing complexity of the nano-sized industrial products and their importance in various fields, 2D-FFF is going to experience a significant growth in the coming years.

Development of optimal microstructured membranes and 2D channel design

The MS membranes fabricated by hot embossing fitted the purpose of “prove-of-concept” as the continuous fractionation of proteins and nanoparticles was successfully demonstrated. Nevertheless, they appear to have several limitations; recovery decreased for the smaller proteins (MW ≤ 150 kDa) and the grooves had round corners. There is a need to optimize the hot-embossing method or to apply alternative methods, such as phase separation [9] and additive manufacturing [10], to develop MS membranes. Porous or non-porous parallel lines could be added on a commercial ultrafiltration membrane to create the grooved pattern using additive technologies such as 3D printing.

As an attempt to explore the option of printing technologies, a feasibility study was carried out at Sirris (Liège, Belgium) employing aerosol jet printing (AJP).
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In AJP, microdroplets of the ink are formed by means of ultrasonication and are introduced on the substrate in the form of aerosol in a stream of carrier gas [11–13]. As a result, better resolution can be achieved compared to common extrusion-based 3D printing systems. Polysulfone (PS) was dissolved in n-methyl-2-pyrrolidone (NMP) and printed over regenerated cellulose membranes (Fig. 6.1). A blue dye was added in the PS/NMP solution to visualize the printed lines. It was demonstrated that the lines were reproducible with a cavity and ridge width of 30 μm and an average height of 10 μm (Fig. 6.1b,c). The RC 5 kDa membrane (Merck Millipore, USA) was rougher compared to the RC 10 kDa (Microdyn-Nadir GmbH, Germany) which did not affect the reproducibility of the lines. However, nowadays printing is still a more time-consuming process compared to hot-embossing.

Figure 6.1 MS membranes fabricated with AJP technology: a) The printed lines are visualized on the surface of the membrane due to the blue dye that has been added to the ink b) 3D surface profilometry of the lines and c) 2D analysis. Left-hand figures: Nadir RC5 kDa, right-hand figures: Millipore RC 10 kDa.
In addition, CFD simulations are a great tool to optimize the 2D-AF4 system; they have been used for several purposes in this study. First, they revealed that the selectivity between the monomer of a protein (bovine serum albumin) and its dimer was higher over rectangular grooves (~2.4) compared to ellipsoidal grooves (~2.1) for the same retention time and therefore a groove structure with sharper grooves is preferred (Fig. 6.2a). Second, the flow profile in AF4 in the presence of the grooves was visualized to gain a better insight on the effect of the aspect ratio of the grooves (Fig. 6.2b). Third, simulations revealed that the modification of the space at the channel outlets was required in the 2D-AF4 channel for the solutes to exit the channel (Fig. 6.2c).

The 2D-AF4 system could be further optimized investigating other channel designs. It is important to note that the 2D-AF4 prototype channel presented in Chapter 5 (Fig. 5.2) was constructed by modifying a conventional FI-AF4 and, for this reason, it was restricted to the original design. For instance, the polycarbonate inlay had an injection inlet in the middle of the channel breadth, and consequently the sample could not be introduced close to the edge of the channel. Last but not least, the groove structure could be optimized by expanding the theory to include the slip length $l_s$ in Eqs. 4.15 and 4.16.
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\[ t_{R,i} = t_{R,i}^{FL} \exp \left( \frac{u_{cr} \cdot (h - l_s)}{D_i} \right) \]  

(6.1)

\[ \alpha = \frac{t_{R,2}}{t_{R,1}} = D_1 = \alpha^{FL} \cdot \exp \left( \frac{(h - l_s)}{\ell_1} (\alpha^{FL} - 1) \right) \]  

(6.2)

**Upscaling of the continuous 2D-AF4 system**

A moderate increase in channel dimensions (length and breadth of the channel) could improve the throughput of the continuous 2D-AF4 system without increasing significantly the footprint. In addition, thicker spacers would allow lower cross-flow rates and consequently higher sample load. This would require an increase in the height of the grooves to retain resolution. CFD simulations could be applied for upscaling. Furthermore, dimensionless analysis could be used for this purpose defining dimensionless parameters that characterize the system. This is something that is already done in AF4 with values such as the retention parameter. The aspect ratio of the grooves, the relative height of the grooves (i.e., the ratio of groove height to the mean layer thickness of the compound) and the aspect ratio of the channel could be used for the upscaling.

It is anticipated that the biggest challenge in the upscaling of the system would be the production of large microstructured membranes. Roll-to-roll printing or roll-to-roll hot-embossing could be used for this purpose.

**Purification of biopharmaceuticals, process control and other regulatory concerns**

A combination of continuous fractionation, optimization of the conditions (carrier liquid, flow rates, etc.), increase in channel dimensions, and use of parallel multilane systems, could lead to unprecedented loading capacity and throughput in AF4 (>1 g/h). This could make it competitive with the current chromatographic technologies. Moreover, continuous systems are more flexible, and it is easier to automate and integrate with other continuous platforms which are available in pharmaceutical industry [14, 15].

However, the transition from lab-scale to production-scale would face many challenges. These challenges would not be limited to the technical difficulties of upscaling that were mentioned above; several regulatory issues may arise. As with other continuous platforms, process validation and process control strategies should be defined and implemented. The process could be monitored by measuring the particle size in the outlets. This could be achieved by sampling and measuring the size distribution with a fast analytical technique, that can give timely measurements such as DLS [16], or by using online process analytical techniques (PAT) that measure properties sensitive to size [17]. In addition, procedures should be established for cleaning and microbial control. In this regard, disposable AF4
channels might be preferred to make sure that the purification is performed under aseptic conditions.

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