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A novel approach to improve operation and performance in flow field-flow fractionation

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ABSTRACT

A new system design and setup are proposed for the combined use of asymmetrical flow field-flow fractionation (AF4) and hollow-fiber flow field-flow fractionation (HF5) within the same instrumentation. To this purpose, three innovations are presented: (a) a new flow control scheme where focusing flow rates are measured in real time allowing to adjust the flow rate ratio as desired; (b) a new HF5 channel design consisting of two sets of ferrule, gasket and cap nut used to mount the fiber inside a tube. This design provides a mechanism for effective and straightforward sealing of the fiber; (c) a new AF4 channel design with only two fluid connections on the upper plate. Only one pump is needed to deliver the necessary flow rates. In the focusing/relaxation step the two parts of the focusing flow and a bypass flow flushing the detectors are created with two splits of the flow from the pump. In the elution mode the cross-flow is measured and controlled with a flow controller device. This leads to reduced pressure pulsations in the channel and improves signal to noise ratio in the detectors. Experimental results of the separation of bovine serum albumin (BSA) and of a mix of four proteins demonstrate a significant improvement in the HF5 separation performance, in terms of efficiency, resolution, and run-to-run reproducibility compared to what has been reported in the literature. Separation performance in HF5 mode is shown to be comparable to the performance in AF4 mode using a channel with two connections in the upper plate.

1. Introduction

Field-flow fractionation (FFF) is a well known family of separation methods that vary in the physical nature of the force field applied to generate separation [1]. Flow field-flow fractionation (F4) [2] is the most popular type of FFF; it employs a hydrodynamic cross-flow, and it exists in several variants: symmetrical F4 [3], asymmetrical F4 (AF4) [4], and hollow-fiber F4 (HF5) [[5], and references therein]. Most recent applications of F4 span from protein to nanoparticle [6–14]. F4 is universally applied to separate macromolecular solutions and particle suspensions based on differences in diffusion coefficient and, consequently, on hydrodynamic size or molar mass within a broad dynamic range (1 nm–50 μm) [3]. Both soluble macromolecules and particulates can be analyzed in one experiment with high resolution (key feature when "free" reagents have to be separated from the fraction that is actually "bound" to functional particles [15]). Because separation takes place without the use of a stationary phase as applied in column chromatography, there is less danger of sample adsorption or physical plugging of the separation channel.

Among the F4 variants, HF5 is the only one that has up to now not been commercially available. HF5 uses a completely different channel geometry based on a polymeric or ceramic hollow-fiber with porous walls as a cylindrical channel.

When a flow is introduced into the channel, it will partly permeate the walls and create a radial cross-flow whereas the remainder will exit the fiber, carrying the sample fractions towards the detector.

Although HF5 has been utilized only by few research groups, the literature shows promising results for protein, nanoparticles, and even whole cell fractionation [17–20]. Very interesting and unique features of HF5 motivate development of this technique for applications in emerging bioanalytical fields such as protein analysis and proteomics, particularly when coupled with mass spectrometry [21–24]. The hollow fibers that have been mostly used in prototype HF5 channels are readily available from manufacturers of water purification cartridges; they are low-cost material, and consequently, allow the construction of a fractionation channel which

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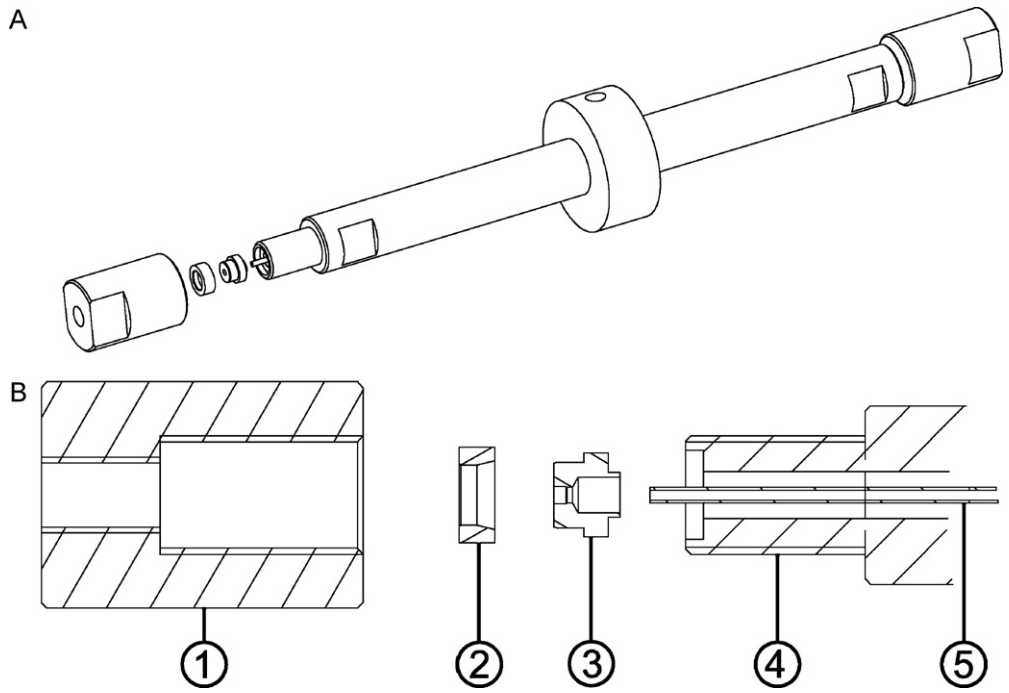

Fig. 1. Scheme of the HF5 cartridge design. It is an assembly (overview A) which consists (exploded view B) of a tube housing of the hollow fiber, with length of 17 cm, the hollow fiber itself (5), two cap nuts (1), two gaskets (3), and two stainless steel ferrules (2) with a inner chamfer on one site. The hollow fiber is sealed with the stainless steel ferrule and the gasket. By tightening the cap nut, the ferrule and the gasket compress the hollow fiber to create a seal against the volume outside the hollow fiber, ensuring that the flow and the sample are introduced into the hollow fiber.

is disposable. A new, fresh membrane could be introduced even after a single run, which is a key feature to avoid run-to-run sample carryover and sterility issues. The low channel volume of typically less than 100 μl reduces flow rates and sample dilution.

To overcome the limitation known from the literature of a tedious manual assembly of the HF5 channel and, even more serious, inferior separation efficiency [25] was the goal of our work.

Another scope of improvement relates to a specific characteristic of the AF4 and HF5 separation mechanism. With these F4 methods the cross-flow is generated from the channel flow, in contrast to symmetrical F4. A so called "focusing/relaxation" step is used, during which flow is introduced from both ends to the channel requiring the flow rate ratio to be adjusted so that the flows meet downstream of the inlet port. Typically one aims at a ratio of 1–9 that is a 9-fold flow rate entering from the outlet port of the channel. The flow profiles at the focusing point are directed perpendicular towards the porous membrane. The sample is injected and transported into the channel through the flow entering the inlet port. Once the sample components have reached the focusing zone, they will be concentrated in a narrow band and at the same time they are exposed to the cross-flow and relaxation is achieved after some time. The correct position of the focusing flow is a critical parameter for a successful separation. Up to now it is commonly verified by injecting a colored sample (e.g. dextrane blue) and observing the focus band through a transparent channel cover plate. In HF5 where the channel cannot be made transparent, visualization is not feasible and flow rates are measured by removing the channel. It cannot be excluded that the effective flow rates during focusing are different or that they change with ageing of the membrane. The improvements suggested in this work provide better control of the focusing conditions, making it easy to place the focus zone at any desired place in the channel and to verify this position in real time for every experiment.

Sample concentration during focusing/relaxation may cause unwanted aggregation or association to higher order species [16]. The developments presented here allow influencing the width of the focusing zone. A wider zone helps to reduce sample concentration and therefore minimizes overloading and sample interactions.

Our aim is to convince more researchers to use F4 instrumentation and prepare the ground for a breakthrough in terms of achieving a significant user base compared to other size-based separation methods, like size exclusion chromatography (SEC). Complexity of F4 instrumentation needs to be reduced and maintenance of F4 channels greatly simplified in order to achieve this goal. The effort to run an F4 experiment should not exceed the skills required for HPLC or SEC.

Here we present for the first time a new F4 system suitable to be used with both AF4 and HF5 channels. The new system equipped with either a two-port AF4 channel or an HF5 cartridge, provides the flexibility to change from AF4 to HF5 mode in the same instrumentation. It is shown that the new design improves the separation efficiency and reproducibility of HF5.

2. Experimental

2.1. HF5 channel design

The HF5 channel reported in Fig. 1 is a construction consisting of a tube housing of the hollow fiber, with length of 17 cm, the hollow fiber itself, two cap nuts, two gaskets, and two stainless steel ferrules with an inner chamfer on one side. The hollow fiber is sealed with the stainless steel ferrule and the gasket. By tightening the cap nut, the ferrule and the gasket compress the hollow fiber to form a seal against the outer volume, ensuring that the flow and the sample are introduced into the fiber.

The cartridge is sealed up to 30 bar (435 psi).

The hollow-fiber material used in the cartridge was polyethersulfone with a nominal molecular weight cutoff of 10 kDa, which corresponds to an average pore size of 5 nm according to the manufacturer; 0.8 mm ID, and 1.3 mm OD (Fiber type FUS 0181, Microdyn-Nadir, Wiesbaden, Germany).