Characterization of Protein and Protein Aggregates using Temperature-controlled Hollow-Fiber Flow-FFF

Trevor Havard, Florian Meier, Evelin Moldenhauer, Soheyl Tadjiki, Thorsten Klein*

POSTNOVA
Leading in FFF

Introduction

Hollow-Fiber Flow Field-Flow Fractionation (HF5) [1,2] is a subtechnique of conventional Asymmetrical Flow Field-flow fractionation (AF4). While AF4 uses a rectangular separation channel, the separation in HF5 takes place in a tubular channel consisting of semi-permeable hollow fibers, which are packed in a respective cartridge. The HF5-cartridge can be used as a disposable in a conventional Postnova AF2000 AF4-system offering fast analysis times, sterile conditions as well as a very good reproducibility. Hence, HF5 represents an excellent fractionation system for e.g., pharmaceutical and biotechnological applications.

AF4 Separation Principle

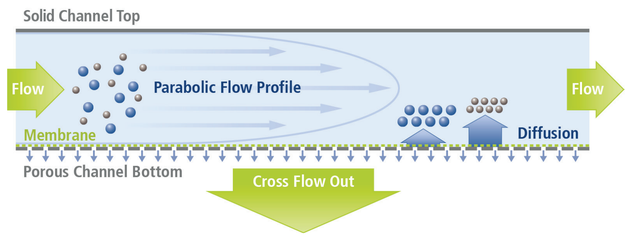


Figure 1: Schematic representation of separation in AF4.

AF4 in Planar and Tubular Channels

Figure 2: Postnova AF2000.

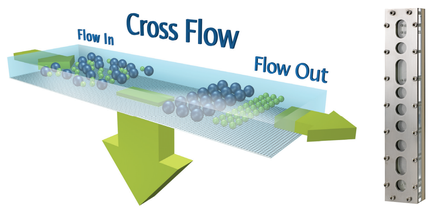


Figure 3: Planar AF4 channel.

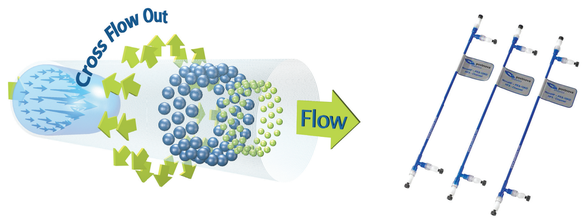


Figure 4: Tubular AF4 channel (hollow fiber).

Reproducibility and Chromatographic Parameters

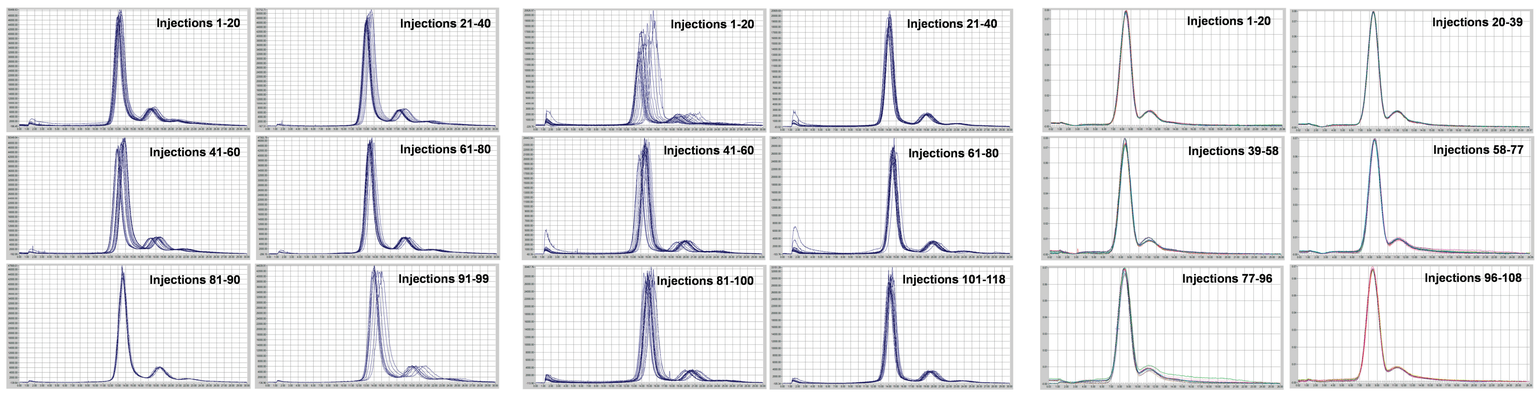
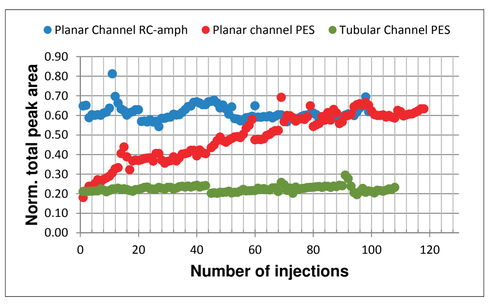
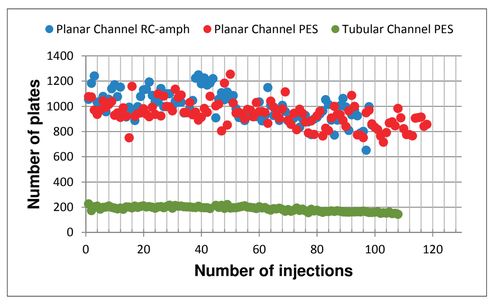


Figure 5: Fractograms of consecutive injections of BSA using the planar channel with the Polyethersulfone (PES) and Regenerated Cellulose amphiphilic (RC-amph) membranes, and a PES tubular channel (hollow fiber).

Channel	*Planar PES*	*Planar RC amph.*	*Tubular PES*	*BSA*
Mean Retention Time [min]	14.43	13.44	8.46	*Monomer*
	19.66	17.99	11.17	*Dimer*
Standard Deviation [min]	0.45	0.43	0.06	*Monomer*
	0.71	0.68	0.09	*Dimer*
Rel. Standard Dev. [%]	3.10	3.17	0.71	*Monomer*
	3.60	3.80	0.80	*Dimer*
Resolution	2.18	1.90	1.35	
Number of Plates	935	1000	157	

Table 1: Comparison of reproducibility, resolution and number of plates of planar and tubular channels.





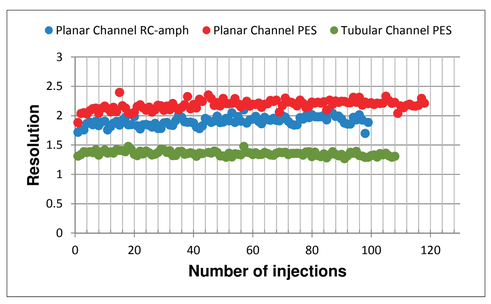
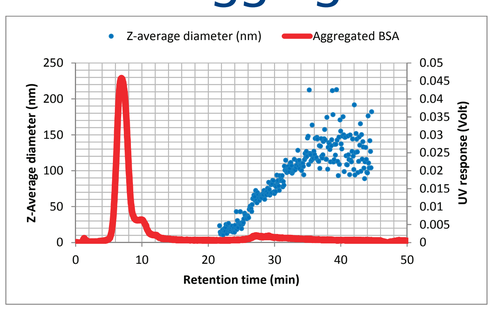


Figure 6: Comparison of number of plates, resolution and total peak area of the planar channel with the PES and RC-amph membranes and a PES tubular channel (hollow fiber).

- Hundreds of consecutive BSA injections
- Tubular channel exhibits better reproducibility
- 3 times higher sample recovery for the planar channels
- 60 % higher resolution and 6 times higher number of plates for the planar channels
- Higher resolution for the applied PES membrane in comparison to the amphiphilic RC membrane

Protein Aggregates



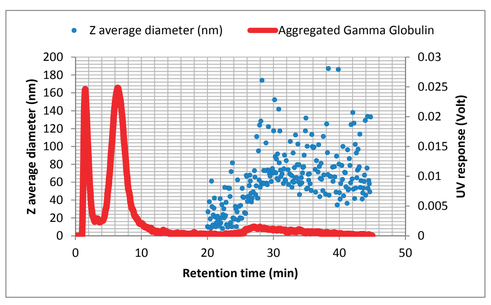


Figure 7: Results of the analysis of aggregated BSA and γ-Globulin samples using a 10 kDa **PES hollow fiber FFF** *system hyphenated with on-line DLS. The BSA sample was filtered through a 0.2 mm filter prior to the analysis.*

Human Plasma

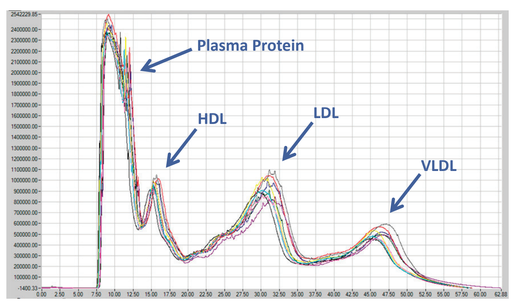


Figure 8: Nine consecutive injections of a human plasma sample using the **planar channel with a 10 kDa RC-amph**. *membrane. The y-axis represents the response of the UV detector at 280 nm wave length. LDL: low density liposomes, VLDL: very low density liposomes, HDL: high density liposomes.*

Conclusions

A temperature-controlled HF5 with focusing ability was assembled by replacing the AF4 channel in the Postnova AF2000 MT system with a disposable hollow fiber cartridge providing less sample and solvent consumption. The planar channel was successfully applied for the fractionation of HDL, LDL and VLDL in a human plasma sample. Hyphenation of HF5 with DLS enabled the successful characterization of aggregated BSA and γ-Globulin at 37 °C revealing the presence of particles in the size range of 20 - 200 nm in both samples.

References

[1] Jönsson JA, Carlshaf A, Anal Chem, 1989, 61(1), 11-18.

[2] Park I, Paeng KJ, Kang D, Moon MH, J Sep Sci, 2007, 28(16), 2043-2049.

Postnova Analytics GmbH
86899 Landsberg, GERMANY
T: +49 8191 985 688 0

Postnova Analytics Inc.
84102 Salt Lake City, USA
T: +1 801 521 2004

Postnova North Europe
01630 Vantaa, FINLAND
T: +358 9 8545 510

info@postnova.com
www.postnova.com