

Separation of rh-GCSF using Asymmetrical Flow Field-Flow Fractionation

Recombinant granulocyte colony stimulating factor (rhGCSF, 18.8kDa) is a cytokine of remarkable therapeutic relevance with a strong tendency towards aggregation. Thus, there is an interest in powerful and convenient separation techniques.

Our aim was to develop an Asymmetrical Flow Field-Flow Fractionation (AF4) method that provides separation and quantification of monomer, soluble and insoluble aggregates and the determination of respective molecular weights (MW). RhGCSF samples containing low molecular weight aggregates (lmwa) and high molecular weight aggregates (hmwa) were produced by applying shear stress or high temperature.

Method development was carried out on an Eclipse AF4 system equipped with the standard channel (25 cm) or the new small channel (18cm), 350 μm or 490 μm spacer and regenerated cellulose membrane (5 kDa cutoff). The samples were analyzed with a DAWN and a UV - detector. Gel permeation chromatography (GPC) experiments were also carried out using the same hardware, but now running in GPC mode using a Tosoh TSKGel® G3000SW_{xl} column.

For the determination of optimal AF4 separation conditions different channels, spacers and channelflow to crossflow ratios were tested. Figure 1 demonstrates that 0.6 mL channel flow with 1.6 mL cross flow (small channel, 490 μm spacer) rendered the best separation. In comparison to the standard channel, the run time could be reduced from 58 minutes to 28 minutes and eluent volume from 251 mL to 42 mL. Finally, MW were determined more accurately with the small channel. For mechanically stressed samples, the MW of the two major fractions could be assigned to a monomer (18.3kDa) and dimer (34.4kDa). Applying the same separation conditions to thermally treated samples the detection of hmwa was feasible in the void peak via the steric hyperlayer mode. Filtered samples which showed a diminished void peak showed this peak to represent hmwa (Figure 2). In contrast, due to the size exclusion, detection of hmwa could not be achieved by GPC. However, good agreement for lmwa was observed (Figure 2).

The successful development of a fast AF4 separation method suitable for a small pharmaceutical model protein and its aggregates was presented. Additionally, in contrast to the standard channel the new small channel technology allowed the determination of the respective MW due to sharper peaks and resulting stronger detector signals.

In summary, a further step towards establishing AF4 as standard analytical tool even for low molecular weight proteins (e.g. cytokines) was taken.

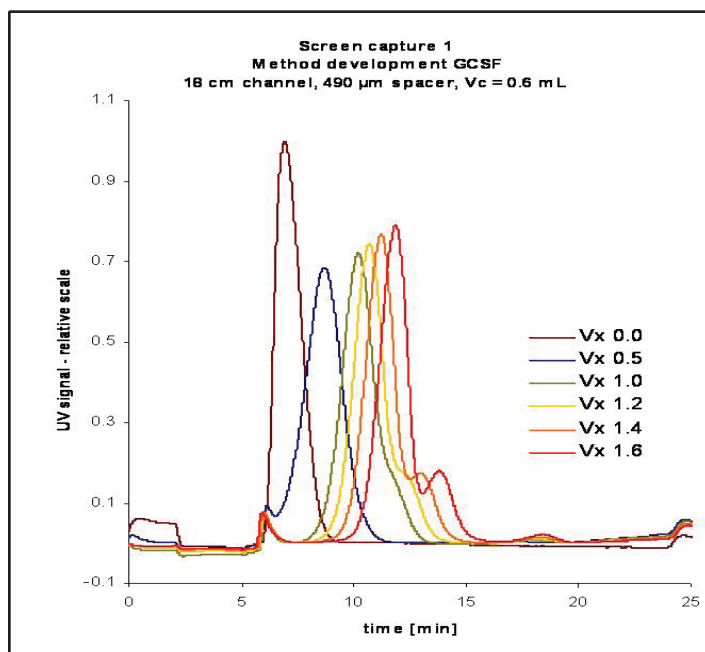


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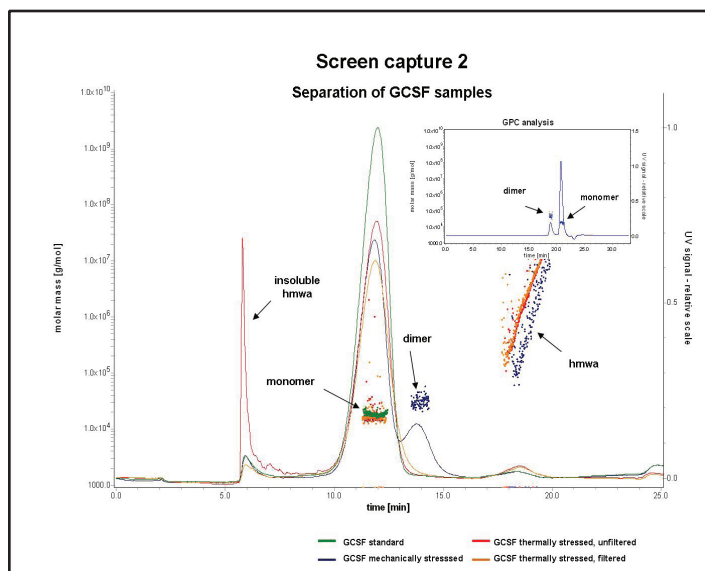


Figure 2. Filtered samples which showed a diminished void peak showed this peak to represent hmwa