

Analysis Conditions / System Set-up

Sample:	Protein-Bacterial Polysaccharide Conjugates
Solvent/Eluent:	0.1 M PBS Buffer, pH 7.2
Cross Flow:	2.7 mL/min
Channel Flow:	1.0 mL/min
Separation System:	Postnova F1000 Symmetrical FFF Series
Detector 1:	Variable Wavelength UV1000 UV/Vis Detector (Wavelength used: 280 nm)

Protein Polysaccharide Conjugates are significant to various areas of biopharmaceutical research and medicine as polysaccharides are known to play an important role immune response of humans and mammals in general.

Symmetric Flow FFF is the premier technique for the analysis of complex and/or sticky macromolecules such as polysaccharides, proteins and aggregates, which cannot be characterized by conventional chromatographic methods. Furthermore FFF can separate the components of the conjugated protein sample, as well as provide hydrodynamic diameter information regarding the proteins, protein-polysaccharide conjugates and aggregates in a single run. By using FFF a complete overview of the sample can be obtained quickly. The following figure shows the separation of such a protein, protein-polysaccharide conjugate and the aggregates.

The figure shows the successful separation of the non-conjugated protein, the protein-bacterial-polysaccharide conjugates, and the aggregates of the conjugates. The mean diameters of the components were determined using absolute calculations based on the unique FFF-Theory developed by Prof. Giddings et. al.. The calculated size of the three major components was 5 nm for the protein, 27 nm for the conjugates and 56 nm for the aggregates.

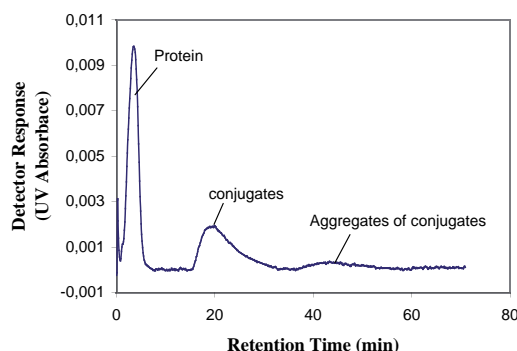


Fig. 1: Fractogram of a Protein-Polysaccharide Conjugate Sample acquired with Symmetric Flow FFF, F1000 CLASSIC.

Why using SF4-UV for Characterization of Polysaccharide-protein Conjugates?

- High resolution separation of proteins, conjugates and aggregates; even a few nanometers can be distinguished.
- Very fast, gentle and nearly interaction free separation without stationary phase also for sticky samples.
- Separation of complex matrices without or with minimum sample preparation or filtering and centrifugation.
- Easy coupling of Symmetric F4 on-line and off-line with many powerful detection systems as UV, RI, LS etc.