NovaSheet

Technology: AF4-UV/Vis

Application: Characterisation of Monoclonal Antibody Aggregates

Analysis Conditions / System Set-up

Solvent	: PBS Buffer, pH 7.2	- Channel Thickness : 250 µm
Injection volume	: 20 µL	- Membrane : NovaRC RCC 10 KD
Sample Concentr.	: 1 mg/mL	- Channel Flow : n.a.
Cross Flow	: n.a	- Focus Time : n.a.
FFF System	: postnova AF2000 Series	- Asymmetric Field-flow Fractionation
UV Detector	: postnova PN3240 UV/Vis	- 4-Channel UV/Vis Detector (Wavelength: 280 nm)

Monoclonal Antibodies are increasingly being used as therapeutic agents. However, the formulation process involved may lead to aggregated forms of the antibody. The activity, biological availability, and possible negative immune responses are directly connected with the existence and generation of aggregates. However, characterization of the monomeric and aggregated from of the antibody is a difficult analytical challenge. Asymmetric Flow Field-Flow Fractionation provides the solution to this characterization need.

Commonly used analytical technologies include static and dynamic light scattering. Batch mode analyses by these techniques determine averaged sizes and the presence of aggregates can be surmised, but quantitation is not possible. The only current technology with any capabilities for protein aggregation is analytical ultracentrifugation. As with FFF, size-sorted species are separated and quantitated for a direct measurement of the size distribution. However, the high cost and need for specially trained users limits the use of AUC to contract and academic institutions. Moreover, collection of the size-classed fractions for elemental analysis or activity determination by, e.g. ELISA, is only possible with FFF.

Asymmetric Flow Field-flow fractionation, AF4, is a chromatographic-like separation technique using an open separation channel instead of a packed column. Lack of a stationary phase provides unique advantages: minimal shear, interaction-free sample characterization, fast separation times, and flexibility with respect to choice of solvent. Also, AFFF offers a wide separation range: from a few nms up to 100 microns. Thus a complete picture of the aggregation phenomenon is provided, including monomer, soluble, and insoluble aggregates. As an elution-based technology, FFF is easily interfaced with sophisticated on-line detectors for multi-dimensional information from a single injection.

For these and additional reasons, FFF is the ideal technology for aggregation studies. AF4 can characterize not only the antibody itself, but also its fragments/aggregates. Furthermore even small differences in conformation and general heterogeneity of sample can be determined.

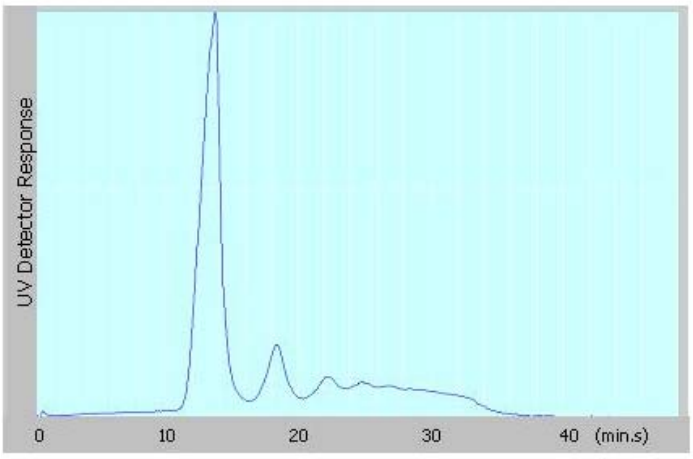

Fig.1: AF4 analysis of an AB sample. The rel. amount of aggregates versus monomer were calculated from peak areas and shown in Tab. 1.

High Resolution, High Recovery. Figure 1 shows the high resolution that can be obtained using the postnova AF2000 system. Table 1 shows the high recovery. In the case of antibody sample preparation 1, the amount of aggregates was also determined by analytical ultracentrifugation (AUC) and Size Exclusion Chromatography (SEC). The AF4 measurement matched the AUC results, but the SEC recovered few of the aggregates, indicating the these species were either screened out by the packing material or degraded by shear forces caused by tortuous flow through the packing.

Table 1. Quantitation of aggregation for various preparations of a monclonal antibody Sample by AF4 with UV detection

Samples	Monomer%	Dimer%	Aggregates	AUC	SEC
Prep 1	59.85	11.23	28.46	24.00	1.00
Prep 2	77.01	3.74	18.69		
Prep 3	95.00	0.00	5.00		
Prep 4	93.07	0.00	7.92		
Prep 5	74.82	13.08	12.35		
Prep 6	70.70	9.00	20.00		
Prep 7	85.71	4.08	8.16		
Prep 8	82.00	3.00	14.00		

Unique Features offered by FFF

Although Asymmetric Flow FFF offers similar information as SEC, the lack of packing material leads to significant advantages for AF4, specifically low shear conditions so that aggregates are not degraded. Versus ultracentrifugation, the AF4 system is easier to operated, easily suited for autosampler use and/or for collection of fractionation. Additionally, the AF4 separation is based on the diffusion coefficient of the various species. Thus, direct measurement of diffusion coefficient and hydrodynamic radius information is also possible.

Why use AF2000 for Antibody Characterisation?

- ► High resolution separation of antibody aggregates.
- ► High recovery of aggregate species.
- ► Fast, gentle and nearly interaction free separation without the use of a stationary phase.
- ► No need to filter sample prior to injection.
- ► Fraction collection and easy direct coupling with further analytical techniques as MALDI, ELISA, etc.

For further information about monoclonal antibody characterization by FFF, please do not hesitate to contact us at: info@postnova.com.