NovaSheet

Technology: AF4 - UV/Vis

Application: Recombinant Antibodies

Analysis Conditions / System Set-up

Sample:	Characterization of Recombinant Antibodies
Solvent/Eluent:	0.1 M PBS Buffer, pH 7.4
Separation System:	Postnova AF2000 Series Assymmetric Flow FFF
Detector 1:	Postnova PN3240 4-CHannel UV Detector (Wavelength 280 nm)

Recombinant Antibodies are widely used in modern drug formulations. Asymmetric Flow FFF (AF4) is the premier technique for the characterization of this kind of antibodies. The main reason for this is, that AF4 can characterize not only the antibody species there selves, but also their fragments and aggregates. Furthermore even small differences in the conformation and the general heterogeneity of samples can be determined.

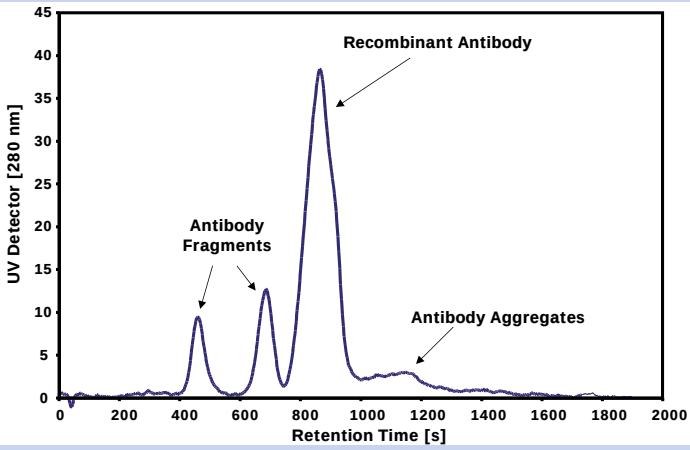

Fig. 1: Characterization of Antibodies with Aggregates and Fragments.

Fig. 1 shows that using the postnova AF2000 system not only the antibody species there selves, but also two fragments and higher aggregates could be separated and characterized. Also the heterogeneity of the antibodies fraction there selves can be shown by using this AF4 system, as the peak shape is not symmetrical but shows a broadening to higher retention times, indicating a different conformation of the antibody species in this region.

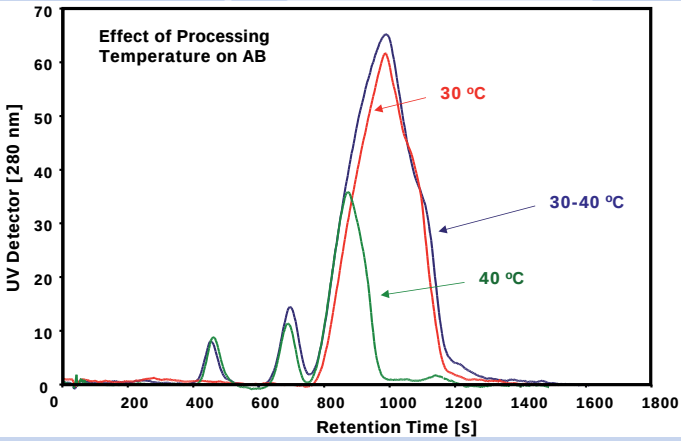

Fig. 2: Effect of different Processing Temperatures on the Antibodies.

Processing Temperature 30°C

Fig. 2 shows the dependence of the antibodies from different processing temperatures. Clearly, the results show, that no fragments and aggregates can be detected when the antibodies are processed at a temperature of 30°C.

Processing Temperature 30 - 40°C

Even at a slightly elevated processing temperature of 30 - 40°C the antibody fraction shows huge changes in its constitution. Two fragment peaks can be detected in the sample. This fragmentation process is caused by the increased temperature. The main antibody peak stays more or less the same, but only shows a slightly increased broadening to higher elution times. That shows that these antibody species with an different conformation are also increasing at 30 -40°C processing temperature. On top of that, some amount of aggregates is generated by the higher temperature, as the small peak eluting right after the main peak of the antibody indicates.

Processing Temperature 40°C

Even more changes in the constitution of the antibody sample occur when the processing temperature is increased to 40°C. Please note that the injection volume was smaller than in the measurements before. The main antibody peak is changing dramatically, as it decreases in size a lot and also the antibody fraction with the different conformation is being decomposed nearly completely. The fractogram shows an antibody peak which is much more symmetric than the peaks discovered before and which is shifted to earlier elution times. This indicates that the conformation of the antibody changed in such a way, that the size is now smaller and the diffusion coefficient is now higher than before. Also aggregates of the main antibody peak can be detected in this sample. But it looks like that the main sample which is missing in the main peak, is deteriorated and has formed the increased amount of fragments which can be found in this sample. So the increased processing temperate is primarily causing a higher amount of fragments than an higher amount of aggregates.

Why use AF2000 for Antibody Characterization?

- High resolution separation of antibody conformation, aggregates and fragments.
- Fast, gentle and nearly interaction free separation without the use of a stationary phase.
- Fraction collection and easy direct coupling with further analytical techniques as MALDI, ELISA, etc.
- Different antibody conformations can be detected.