Monoclonal Antibodies and the Preparative Use of Flow Field-Flow Fractionation (AF4)

Proteins—especially monoclonal antibodies (MABs)—have become increasingly important in pharmaceutical work. However, there are some important differences between conventional, chemically-synthesized drugs and proteins. Because of the complex and weak structure of proteins, even a slight change in conditions, such as pH value, temperature or mechanical stress, may lead to aggregation and a loss of activity and/or stability.

A separation of MABs before analysis is preferred because a detailed investigation of a fraction would be much easier and provide a better insight into the physical and chemical properties, compared to an analysis in the presence of a coexisting monomeric protein.

Asymmetrical flow field-flow fractionation (AF4) is a well established method for sizing and quantifying different aggregate species in protein formulations. A major advantage of AF4 is the use of the formulation buffer of the protein as the mobile phase. The separation is independent of the ionic strength of the buffer.

The capability of AF4 as preparative tool for the separation of aggregates, fragments and monomeric species was investigated in this study. The system consisted of an Eclipse (Wyatt Technology Europe GmbH, Dernbach, Germany), equipped with DAWN multi-angle light scattering (MALS), refractive index (RI) and ultraviolet (UV) detectors, and a fraction collector. The separation was achieved by using a semi preparative SP channel with 350 µm (height).

A monoclonal antibody sample was exposed to light, resulting in the formation of fragments and aggregates [Fig. 1]. One mg of protein per run was injected into the channel and collected in tubes made of glass, using a Gilson FC-203B fraction collector. The collected fractions were concentrated and the final concentration was determined by UV absorbance at 280 nm or using a Micro-BCA Assay.

AF4 is a useful method for analytical and preparative separation of protein aggregates since the separation can be performed in each buffer or even pure water, thus facilitating subsequent activities. Furthermore, an investigation of possible immune responses triggered by protein aggregates in comparison to monomeric proteins can be performed, which is currently of major interest in administrative organizations and the pharmaceutical industry at large.

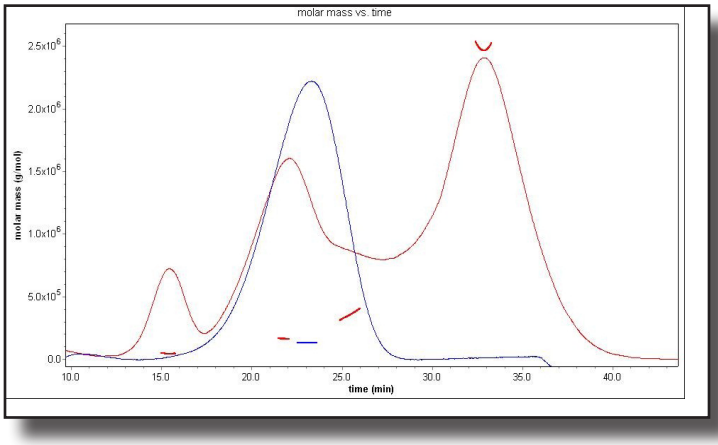

Fig. 1. UV 280 nm fractograms and MALS detection of light exposed MAb1 (red line) and native MAb1 (blue line).

Peak Number	Molecular Weight	Protein Species
Peak 1	37 kDa	Fragments
Peak 2	166 kDa	Monomer
Shoulder	295 kDa	Dimer
Peak 3	2770 kDa	Oligomer

Table 1: Molecular weight of the detected species of MAb1

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