

Quantification of Insoluble Monoclonal Antibody Aggregates

From a quality and safety perspective it is critical to prove that a protein drug product is free from high molecular weight aggregates (HMW). These species cannot be detected by GPC due to their size (> 200 nm). Thus, as an unspecific standard method in pharmacopoeias, light blockage, is frequently used. This method counts particles $\geq 1 \mu\text{m}$ without quantifying the actual ratio of HMW aggregates to monomer. Furthermore, light blockage measurements need large sample volumes and individual dilution regimes.

In 1993 Litzén *et al.* demonstrated that by applying asymmetrical flow field flow fractionation (AF4), HMW monoclonal antibody aggregates eluted in the void peak via the steric-hyperlayer mode. The aim of this investigation is to propose a method for detection, separation and quantification of monomer, soluble oligomers and aggregates and insoluble antibody precipitates in a single run.

A monoclonal IgG antibody was subjected to AF4 and light blockage analysis. HMW aggregates of the antibody were generated by severe pH stress, rendering a turbid precipitate suspension. Different amounts of this suspension were admixed to a standardized amount of antibody monomer (0% - 10% precipitate). Filtered samples, imitating GPC samples, and unfiltered samples, were compared.

We found that #1. Chromatograms of filtered and unfiltered samples differ in the AF4 void peak only, demonstrating that monoclonal antibody species which are not detectable by GPC are present only in the void peak (Fig. 2, Screen capture 1). And, #2. The intensity of the void peak of unfiltered samples increases proportionally with increasing amounts of HMW aggregates, allowing quantification (Fig. 2, Screen capture 2).

A comparative analysis of the samples by light blockage showed an analogous proportional increase in particle numbers (Fig. 1).

Our studies prove that AF4 is capable of quantifying specific amounts of HMW monoclonal antibody aggregates via the steric-hyperlayer mode. This is possible using extremely small sample volumes (~ μL) and does not need individual dilution regimes. Thus, AF4 can uncover HMW aggregates on biopharmaceuticals, in one single sample run alongside with low molecular weight impurities.

The analyzed samples did not contain noteworthy amounts of soluble aggregates because the applied stress, as noted before, did not induce such aggregates, and the monomeric standard itself was nearly free of impurities. If these samples had been analyzed by GPC, the product would have appeared to have been "clean", which is untrue in this case.

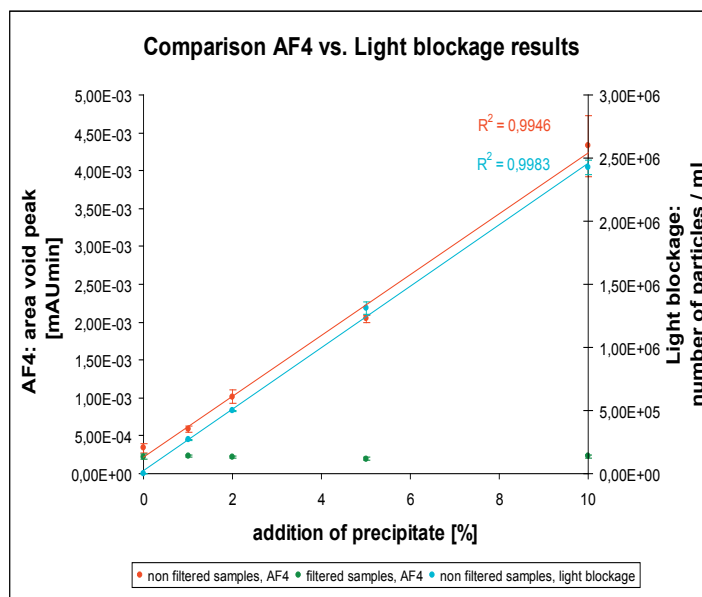


Figure 1: AF4 and light blockage results

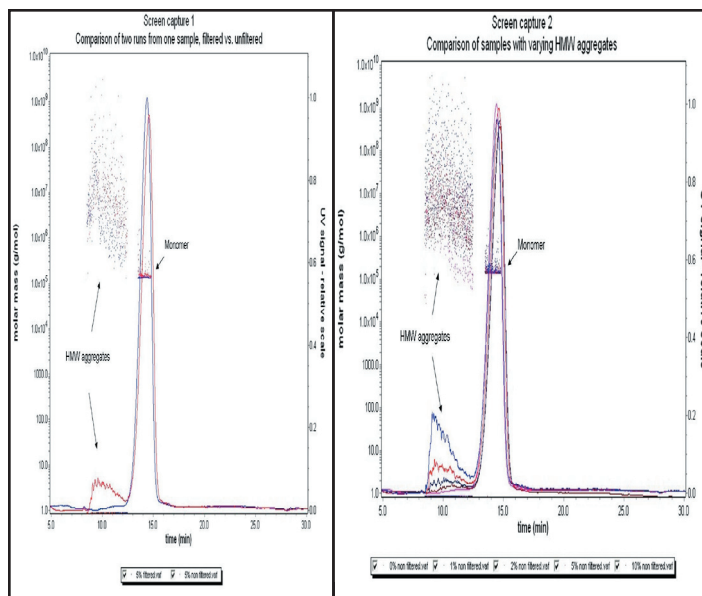


Figure 2: Aggregates only shown in the void volume of the screen capture 1; and increasing aggregation in screen capture 2.