

Protein Aggregate Removal with the DynaPro DLS Plate Reader

The presence of aggregation in immunoglobulin biopharmaceuticals is a significant concern during all phases of drug development. With increasing monoclonal antibody titers produced in bioreactors, manufacturers are seeking methods and processes to effectively remove aggregates. Analytically, a high throughput aggregate detection method would speed up the evaluation and validation process during aggregate removal implementation. To evaluate the aggregate removal capability of a purification procedure, an IgG feed with extremely high aggregate levels was generated, and the feed was passed through a purification device in flow through mode under two different buffer conditions. The feed, flow-through fractions, and regeneration samples were analyzed.

Figure 1a represents the species population for sample set 1 obtained by SEC-MALS analysis. The feed sample was observed to contain 39% monomer, 16% dimer and 45% high molecular weight aggregates (HMW). For the flow through samples, monomer and dimer species were detected in the earlier fractions (Fractions 1-6), while HMW aggregates began to appear in the later fractions. The regeneration peak contained mainly HMW species, accounting for around 90% of the population.

These results demonstrate that the breakthrough of non-monomer species occurred as early as fraction 1. Consistent with these observations was an immediate increase in average hydrodynamic radius (R_h) from fraction 1 to fraction 2—and was detected by DLS (Figure 2, experiment 1). This indicated the early breakthrough of non-monomer species in fraction 2, if not fraction 1. The sudden increase in the average hydrodynamic radii from fraction 5 to fraction 6 also indicated the sudden increase in aggregates (dimers or HMW species). The measured R_h for the regeneration sample is 18.6 nm—even greater than the value of 16.3 nm for the feed sample, indicating the HMW population is greater than that for the feed sample.

When we plot the percentage of aggregates obtained from SEC-MALS vs. the average R_h measured by DLS (Figure 1), excellent correlation was found for the flow through fractions. Although DLS can not provide the species distribution as can be obtained by SEC-MALS, it is a quick analytical tool to identify conditions that effectively separate aggregates from monomeric IgG. As shown in Figure 2, it is clear that the operating condition in experiment 1 achieved better separation than condition 2.

In summary, DLS served as a quick reliable analytical tool to optimize the operating condition for aggregate removal. Our bioanalytical laboratory receives large numbers of requests to evaluate aggregate levels. Completing the data acquisition for a set of 96 samples with SEC-MALS would have taken 32 hours not including sample preparation, HPLC set up and final data analysis. The installation of DynaPro Dynamic Light Scattering (DLS) plate reader greatly shortened the data acquisition time and improved Millipore's assay capabilities.

This note graciously submitted by Ying Wu, Millipore, Bioprocess Division, Bedford, MA 01730.

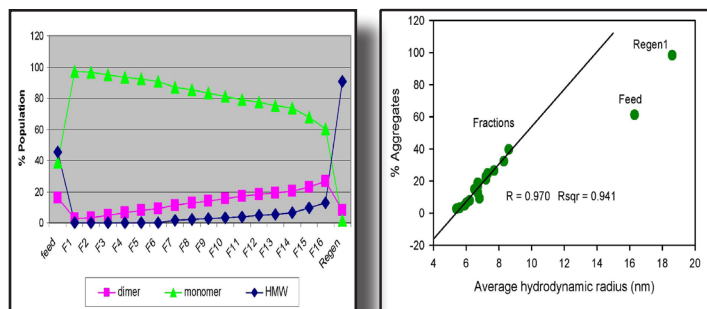


Figure 1: Flow through profile of monomer, dimer and HMW species obtained by SEC-MALS (upper left) and the correlation plot of percentage aggregates by SEC-MALS vs. average hydrodynamic radius measured by DLS (upper right).

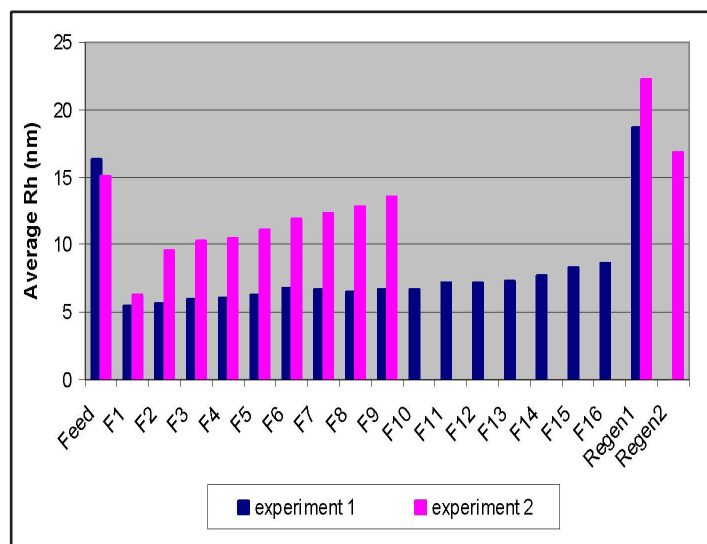
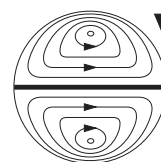


Figure 2: Breakthrough curves for Experiment 1 (in blue) and Experiment 2 (in pink) obtained by DLS.



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