

Rapid, Autonomous Lot-to-Lot Comparability of Monoclonal Antibodies with the Calypso

Analysis of purity and demonstration of lot to lot comparability is essential throughout the drug development process. Among the various biophysical and biochemical assessments of purity to which biological therapeutics are subjected, one of the most fundamental is based on the molecular weight of the species present in aqueous solution.

A common approach to evaluate the relative levels of molecular weight forms is to perform analytical size exclusion chromatography (SEC) in combination with multi-angle light scattering (MALS) and absorbance (UV) and/or refractive index (RI) concentration detectors. For monoclonal antibodies, a dominant peak (>95% total peak area and molar mass consistent with an intact antibody) along with a small number of minor high and/or low molecular weight species is frequently observed. While SEC-MALS data are robust and precise, the method is not without potential concerns. Sample dilution, weak non-specific interactions with the column, shearing, or exclusion of high molecular weight species are frequently cited effects that may impact the complete analytical characterization of purity based on size fractionated material.

To validate SEC-MALS characterizations, orthogonal experiments are performed that specifically address the issues surrounding use of an SEC column. In one such experiment, a series of dilute, non-fractionated samples are directly injected into the concentration and MALS detectors. A general agreement between the molar mass calculated from this batch mode and the molar mass calculated from SEC-MALS provides assurance that the SEC-MALS analysis is reflective of the distribution of species in the non-fractionated solution. Although conceptually straightforward, in practice the batch MALS technique is both time and material intensive. Manually acquiring data for two samples is labor intensive and often requires a full day, or more. The Calypso system is perfectly suited to automate these experiments, significantly both the hands-on experimental time and amount of sample consumed.

Two separate production lots of a monoclonal antibody (mAb1; Regeneron Pharmaceuticals, Inc.) were characterized in the batch mode using the Calypso instrument (Wyatt Technology). Each mAb sample was diluted from a stock solution to a concentration of ~0.8 mg/mL in 0.02 μ m filtered phosphate buffered saline (PBS, pH 6.8). Alternatively, the protein can be dialyzed against buffer if the stock concentration is relatively low. Filter membranes were removed from the flow path in order to prevent altering the sample; however, the filter housings were kept in-line to act as particulate filters (5 μ m PAT frits). The automated Calypso method consisted of two separate protein concentration step-gradients, one for lot

1 (Pump 1) and a second for lot 2 (Pump 2) with a buffer (Pump 3) wash step in between (Figure 1). Each dilution series consisted of five protein concentrations (0.6 – 0.2 mg/mL, in 0.1 mg/mL steps) for each lot, requiring less than 7 mL (5.6 mg) of diluted protein. A 90 second delay was programmed after each injection for data acquisition. Step gradients were delivered to an online miniDAWN TREOS and T-rEX differential refractive index detector with data collected in Calypso and ASTRA software, simultaneously.

In the dilute limit (i.e. protein concentration less than 1 mg/mL), light scattering is directly proportional to protein molecular weight and does not require a virial expansion to account for non-ideality. Figure 2 shows a close linear relationship between the 90° light scattering signal and differential RI signal, indicating a near monotonic molar mass across all concentrations. Data analysis was performed with ASTRA6 and the calculated molar mass from the plateau region of each injection are shown in Figure 2. The molar masses determined with Calypso Batch MALS data agree well with those determined from SEC-MALS (151.5 kDa, data not shown), indicating the SEC column did not significantly affect the sample.

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Figures for Rapid, Autonomous Lot-to-Lot Comparability of Monoclonal Antibodies with the Calypso

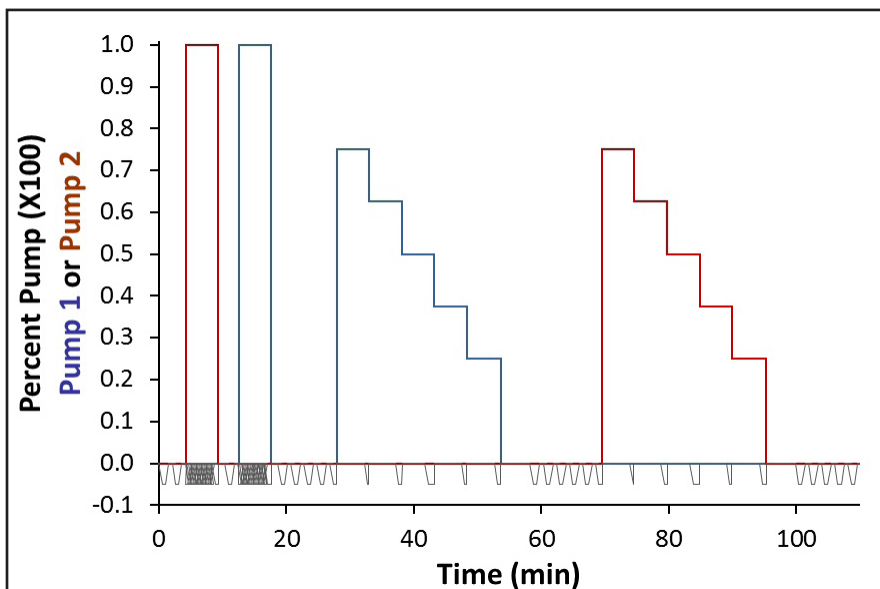


Figure 1: Method schematic for automated batch MALS comparison of two lots of monoclonal antibody. The syringes and flow paths for Pump 1 and Pump 2 are first saturated with the respective protein samples (0-20 minutes) followed by the two separate concentration gradients (~30-50 minutes and ~70-90 minutes). Buffer injections (Pump 3) are programmed between the series of protein injections to restore the baseline. The samples were prepared at a higher concentration so that all injections require mixing of protein and buffer.

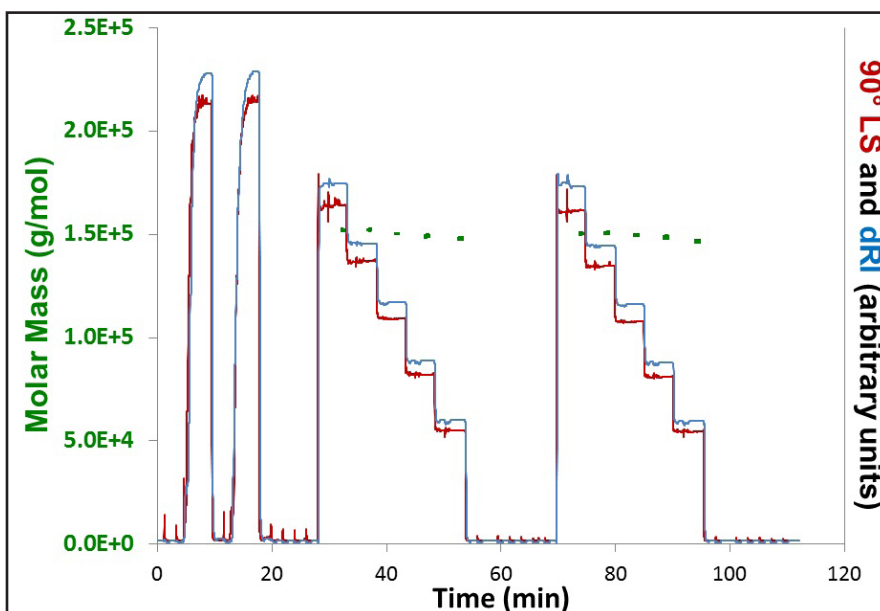


Figure 2: Raw light scattering and differential refractive index signals (continuous red and blue traces, respectively) and calculated molar mass for each of five injected concentrations per lot (green data points). Molar masses were determined in ASTRA6 utilizing data from all three light scattering detectors within the delay periods. The average molar masses were 150.2 (± 2.0) kDa and 149.0 (± 1.7) kDa for mAb1 lot 1 and lot 2, respectively.



The Calypso Composition Gradient System



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