Rapid assessment of Aggregation of Biologics in different matrices and from Upstream and Downstream Development Process Stages

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Abstract

Assessing and controlling aggregation of biologics is vital to ensure safety and efficacy of a biopharmaceutical product. Aggregation has been recognized as a major issue for modern therapeutic modalities such as bispecific antibodies and Fc fusion proteins. Therefore, checking for aggregation propensity is an essential part of developability assessments throughout the whole development cycle, starting from in-silico approaches in the protein design phase to experimental confirmation and analysis in the discovery and upstream and downstream development stages. In these stages, hundreds of samples need to be analyzed and these numbers pose a big challenge for current analytical approaches (mainly size exclusion chromatography, SEC), which are slow and require sample purification. We have addressed this bottleneck by developing a high throughput aggregation screening assay which allows assessment of aggregation of molecules containing the antibody Fc domain in different sample matrices and cell culture supernatants. This assay is the first assay which is capable of screening hundreds of samples of samples within two hours and without purification of samples. We are presenting different data sets from forced aggregation studies as well as data from Biopharma development partners.

Results

The Aggregation Assay for Fc containing proteins (Article No. PA-301) has to meet the needs of analytical development scientists to be a real alternative to SEC. Hence, it needs to be a robust, quantitative method with high accuracy, precision and linearity.
The PA-301 assay yields the product-related aggregation content in form of a relative fluorescence unit, the so called net signal. The assay workflow and the required data analysis are not covered in this work and can be requested from PAIA Biotech.

Accuracy, precision and linearity

Internal data from PAIA Biotech's quality control samples (IgG1 with forced aggregation) in **Figure 1** shows correlation of the PA-301 assay to SEC analysis with $R^2 > 0.99$. The calibration curves show average CVs < 7 % within triplicates and a linear dynamic range from 1~30 % HMWS with an accuracy within ± 15 %, complying with the EMA analytical method validation guidelines[1] (data not shown). Furthermore, it is worth to mention the Lot-to-Lot stability across multiple produced assay kit batches, making for a good foundation for an orthogonal analytical method to SEC.

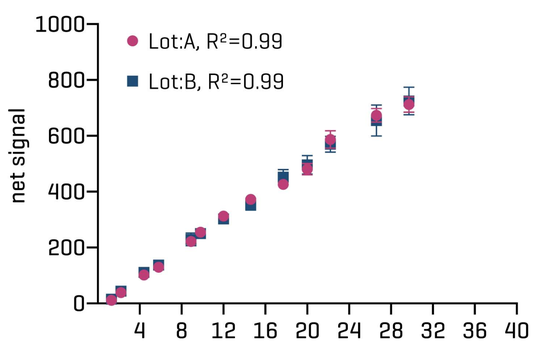

Figure 1. Calibration curve of the PA-301 assay. IgG1 was force aggregated and run in the PA-301 assay with two different assay kit batches. Accuracy, precision and linearity is similar for both batches and comply with typical method validation guidelines across a large, representative dynamic range for Fc containing biologics.

There are multiple possible applications of this assay for the biopharma industry: From developability assessment in Discovery, over cell line selection in Cell Line Development to process characterization and optimization for Upstream and Downstream Development.

Developability assessment

A common procedure in the developability assessment of novel biologics is application of several stress conditions, like freeze/thaw cycles, sheer stress, pH stress or thermal stress. **Figure 2** shows three different mAbs under low and high thermal stress ranging from 70-80 °C at various incubation times. Low and high temperature stress led up to 10 % and up to 80 % HMWS, respectively, according to SEC analysis. Although these are different mAbs they show similar and linear net signals when plotted against the HMWS % values from the SEC analysis. This poses a benefit for discovery work where e.g. different lead candidate sequences can be compared with each other to assess the aggregation propensity and identify the most promising ones.

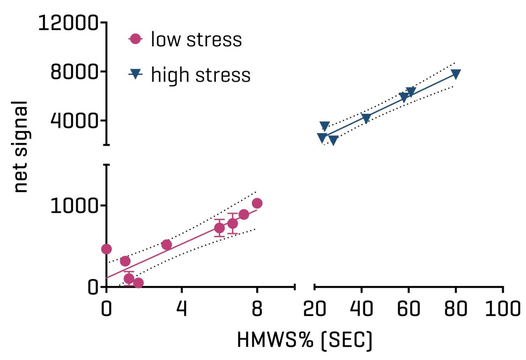

Figure 2. Application and evaluation of thermal stress on monoclonal antibodies. Different mAbs were stressed from 70-80 °C at various incubation times. Some conditions let to rather minor aggregation ("low stress") and other conditions led to high aggregation contents ("high stress") according to SEC analysis. These samples were also run in the PA-301 assay and showed similar rankings with low offset between the different mAbs.

Aggregation analysis is a method which is traditionally being applied only after affinity purification of given biologics of interest. The reason for this is that it is neither practical (HPLC column quality loss) nor sufficiently accurate to perform a SEC run directly from crude cell culture supernatant. However, it is of high interest to analyze the aggregation content before purification of the biologic especially with increased work on novel modalities (bsAb, Fc fusion, etc) to biopharma's R&D. These novel modalities are often more aggregation prone than traditional mAbs.

Since affinity purification can be an expensive and time-consuming process step for many proteins, it is desired to have a solution which can reliably determine the product-related aggregation content directly from cell culture supernatant, ideally without any sample preparation and quick turnaround time. With the PA-301 assay it is now possible to screen for product-related aggregation without the need of prior affinity purification of the biologic, cutting costs and timelines. It also enables a new layer of quality check in Cell Line or Upstream Process Development, allowing to screen more samples than previously.

Cell culture supernatant screening

A possible way to reduce aggregation in cell culture is to systematically alter the cultivation parameters according to principles of Design of Experiments (DOE). The goal of a study like this would be to identify parameters which do not significantly lead to increased levels of aggregation.
Figure 3 shows the impact of two factors, Medium and Feed, on the aggregation content of Trastuzumab samples in a Fed-Batch DOE. After Protein A purification of all samples with subsequent SEC analysis it can be shown that Medium 1 and Feed C yield the lowest aggregation content with around 3 % HMWS (**Figure 3 A, B**). DOE analysis was performed with Design-Expert 13 (StatEase) and showed that Medium and Feed impact aggregation independently. According to this analysis the cultivation temperature was not affecting the aggregation content (data not shown). As the PA-301 assay also allows for analysis of crude cell culture supernatant, the supernatant samples were diluted 1:6 in 1x PBS buffer and run in the assay without any other sample preparation. The PA-301 assay also identified Medium 1 and Feed C as the ideal setpoints to reduce aggregation (**Figure 3 C, D**). Therefore, in this study it would not be necessary to purify the samples and analyze them by HPLC, reducing timelines to draw similar conclusions regarding the aggregation behavior in cell culture. However, the model with the results from the SEC analysis better describes the variance for the range of 2.5~3.5 % HMWS than the PA-301 assay, with a model $R^2=0.85$ vs. $R^2=0.46$, respectively. This illustrates that these aggregation levels in cell culture supernatant screening applications are close to the limit of detection of the PA-301 assay.

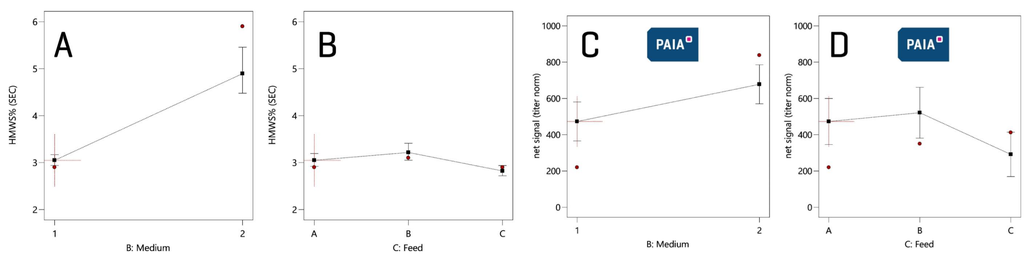

Figure 3. DOE evaluation of Fed-batch culture. The DOE for this Trastuzumab expressing Fed-batch consisted of 15 ambr15 runs (performed by SELEXIS). It was a custom design with three temperature levels (33, 34, 35 °C), two different medium levels (1, 2) and three different feed levels (A, B, C). Design-Expert 13 (StatEase) has been used to evaluate the data via multiple linear regression. Changes in medium and feed were correlated with the HMWS% content measured by SEC (**A, B**) and with the net signal measured by the PA-301 assay (**C, D**).

Downstream Process Development

It is obvious that the assay accuracy and sensitivity are better if the samples are purified.
Therefore, Downstream Process Development is another application area for the PA-301 assay. While going down through "purification funnel" with multiple chromatography steps, it is necessary to monitor product-related impurities like charge variants, fragmentation and aggregation. Here we show data from different mAb samples after the first Protein A purification step. The samples were either purified with a Citrate or Glycine based buffer.
An aliquot of formulated Pertuzumab was additionally stressed at 75 °C for 30 min as a high aggregation control. The samples were analyzed by SEC and with the PA-301 assay in parallel. The PA-301 was calibrated with a Trastuzumab calibration series (data not shown). The HMWS% content from both methods correlated with $R^2=0.89$ although the PA-301 assay data has a certain offset, displaying higher HMWS% values than expected (**Figure 4**). Both methods revealed that the Glycine elution led to higher aggregation compared to Citrate elution. Hence, as the PA-301 assay is a very fast analysis method, it incentivizes larger screening DOEs for elution buffer compositions to keep the aggregation content in the first purification step as low as possible and to unburden later DSP stages.

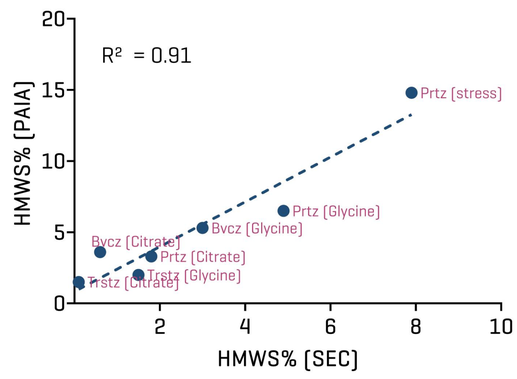

Figure 4. Aggregation analysis of different mAb after Protein A purification. Bevacizumab (Bvcz), Pertuzumab (Prtz) and Trastuzumab (Trstz) were expressed in Fed-batch mode and subsequently purified via Protein A HP SpinTrap columns (Cytiva) (performed by ProBioGen). For purification, two different elution buffers were used to assess the impact on aggregation of the elution buffer. As a high aggregation control a formulated Pertuzumab has been stressed at 75 °C for 30 min.

Conclusions

The Aggregation Assay for Fc containing proteins (PA-301) has proven to be a robust, quantitative method with high accuracy, precision and linearity. It gave similar results compared to size exclusion chromatography (SEC) in forced aggregation studies. It is possible to analyze Fed-batch samples for optimization of aggregation on diluted cell culture samples. And lastly, it has been successfully shown its usefulness in affinity purification studies.
This work adds up to recent PA-301 data on aggregation prone molecules like bsAbs and Fc fusions (data can be requested from PAIA).

References

[1]https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf (2022-06-21)