Application Note 1 – Introduction to the Proteometer-L Kit

BACKGROUND

The Proteometer-L Kit is designed for rapid analyses of intact IgG and IgG fragments containing the Fc region in harvested Cell-Free Culture Filtrate (CFF). The kit measures mAb titer and relative aggregate content, without the need for sample purification.

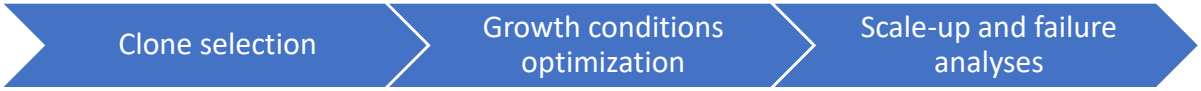

Fig. 1. Steps in the early development of therapeutic mAbs

Samples from many small fermentors are compared for clone selection and optimization of growth conditions in the early development stage of therapeutic mAbs (t-mAbs, Fig. 1). mAb content (titer) and aggregate profiling in CFF constitute a large fraction of the analyses performed at this stage. mAb titer is monitored since it determines the yield of the final drug substance. Aggregate content is a Critical Quality Attribute (CQA) of t-mAbs that can affect its efficacy and safety. It is monitored throughout process development and manufacture. Aggregate analyses, even in CFF, give some indication of the propensity of the t-mAb to form aggregates and is measured early during development for risk assessment.

The Proteometer-L kit offers the following advantages over existing methods for the determination of mAb titer and aggregate content:

- Two critical quality attributes in one assay
- CFF can be directly analyzed (no additional purification needed)
- Linear response from 0.5-16 mg/mL mAb
- Very small sample volume required, minimum 0.5 µL
- Fast – 10 min run time

PRINCIPLE

Size Exclusion Chromatography (SEC) is the most widely used method for the assessment of size variants such as aggregates in t-mAbs. Small aggregates such as dimers, trimers, and tetramers, as well as monomers and fragments, are detected and quantified by monitoring the absorbance of the purified t-mAb analyte at 280nm. CFF cannot be directly analyzed by this method due to interference by proteins and metabolites at this wavelength.

In the early development stage, purification of mAb or IgG from CCF using Protein A is typically the first step. Often this is followed by SEC for aggregate profiling and ion exchange chromatography for many analyses of charge variants. Therefore, these separation modes make up a substantial portion of the analysis performed during mAb development.

The most widely used LC-based method for mAb titer determination is Protein A affinity chromatography. Unlike traditional method the Proteometer L assay requires as little as 0.5 µL of CFF per assay, thus allowing multiple analyses from even 15 mL fermentors.

The Proteometer-L assay can be used to quantify proteoforms of intact human IgG molecules of IgG1, IgG2 and IgG4 subclasses. It is therefore suitable for the analyses of the vast majority of t-mAbs. The assay is based on the specific fluorescence detection of a conserved Fc domain on IgGs by molecular recognition. The multitude of non-mAb CFF components are not detected, allowing mAbs to be analyzed directly from the fermentor. Both mAb titer and relative aggregate content can be quantified in one run.

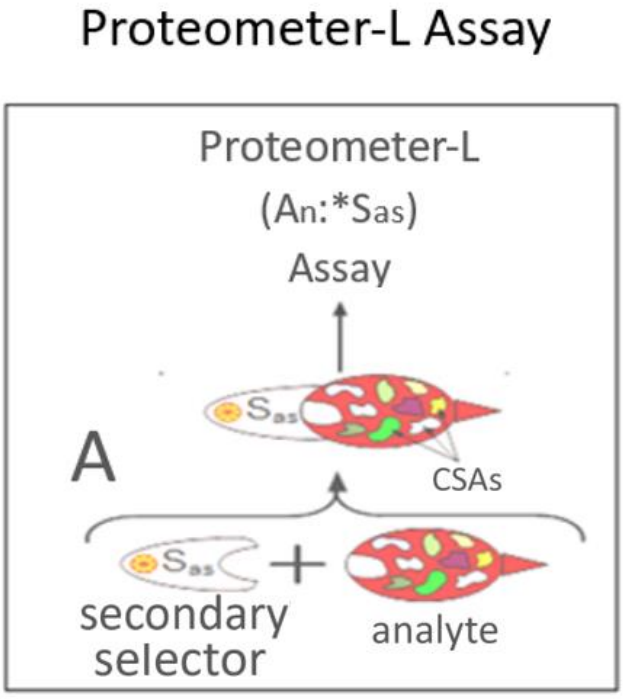

Figure 2. Proteometer-L Assay

EXPERIMENTAL

The method was tested on two Shimadzu HPLCs:

- Nexera 2 with a quaternary pump, 40 µl mixer
- LC-40 with a SIL-40C XR autosampler, LC-40D XR quaternary pump, CT40C column oven, RF-20AXS, fluorescence detector, and an SPD-40 PDA detector

The dead volume on both systems was less than 40 µL

Method parameters were as follows:
Flow rate: 1.0 mL/min
*Mobile Phase: PROTEOMETER-L MP
FLD Wavelengths: Ex 450 nm, Em 520 nm
**FLD Detector settings: Gain 4, Sensitivity Medium
**Injection Volume: Variable, 0.5-16 µL
Run Time: 10 min
Novilytic Proteometer Reactor Column, 7.8 x 150 mm

*Note: Mobile phase was kept in low actinic glassware
**Note: Both can be individually adjusted depending on the mAb concentration in the sample to achieve or optimize the desired result

The method was linear between 1-16 µg of mAb injected:

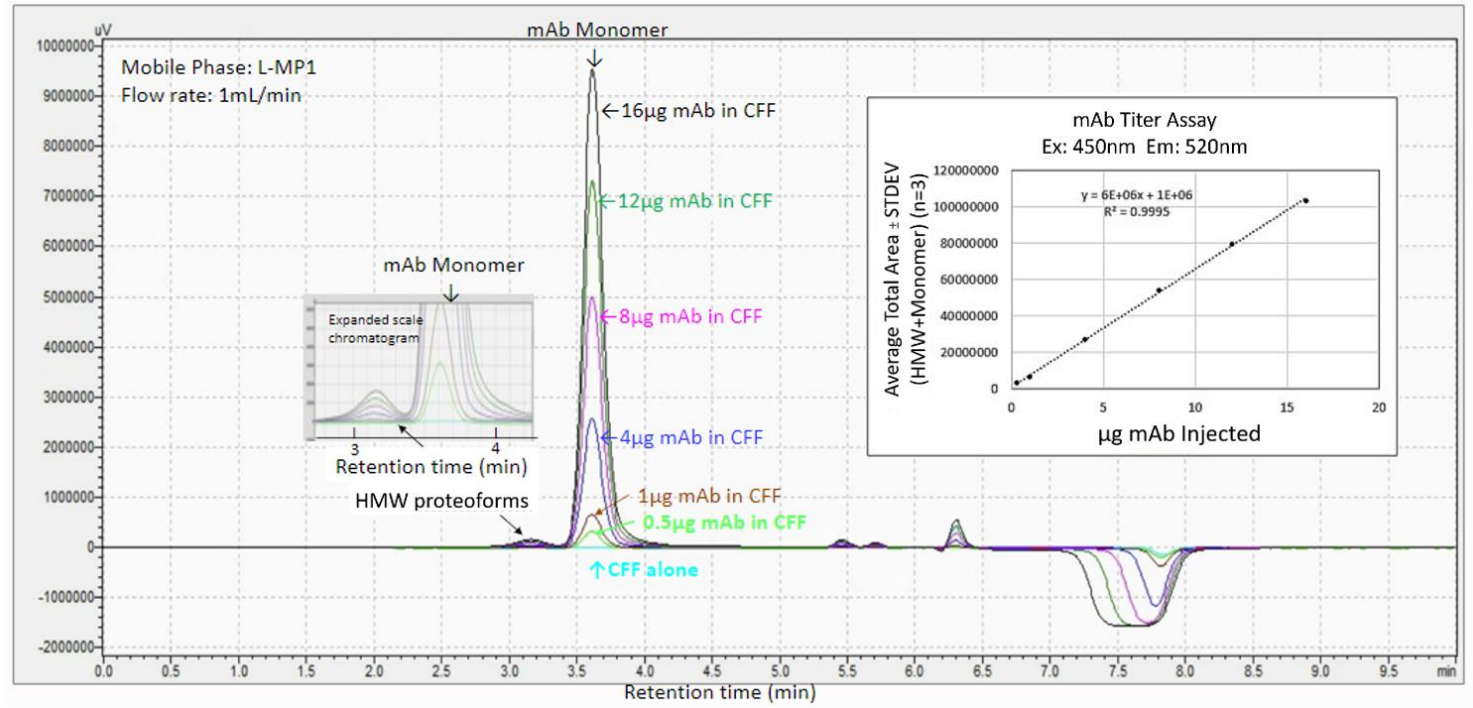

Figure 3. Proteometer-L Linearity

mAb Titer and Aggregate Content was robust over 3 days:

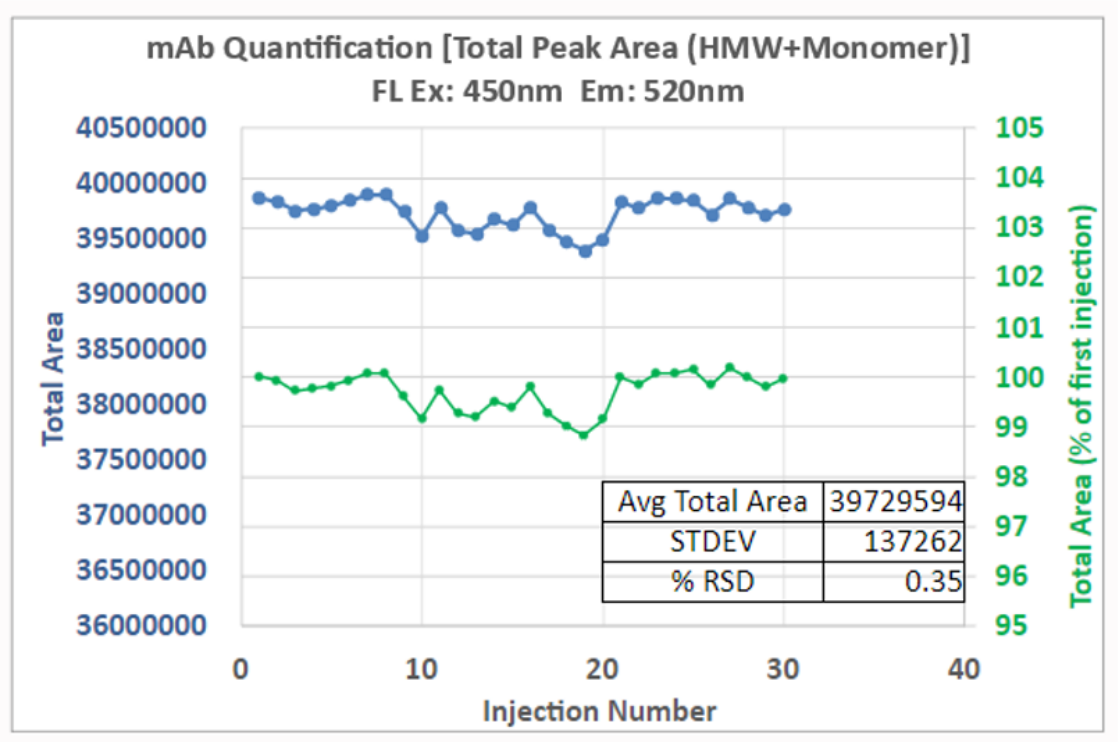

Figure 4. Proteometer-L Total Peak Area Repeatability for 30 Injections over 10 Days

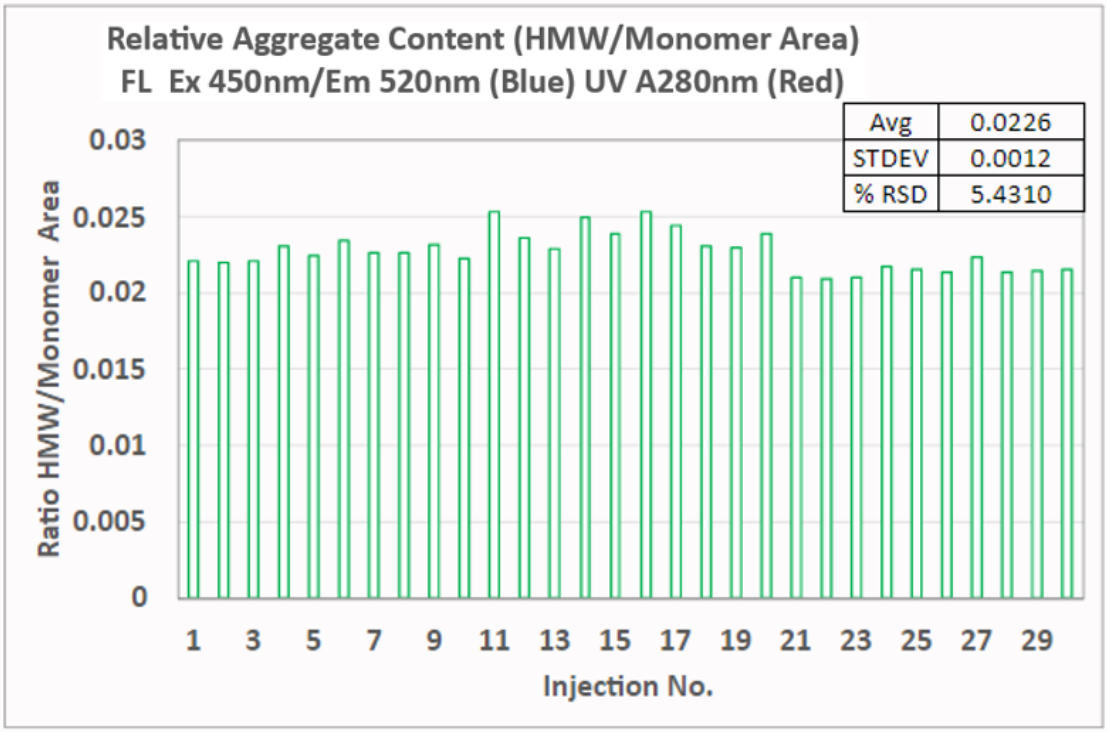

Figure 5. Proteometer-L Relative Aggregate Content Repeatability for 30 Injections over 10 Days

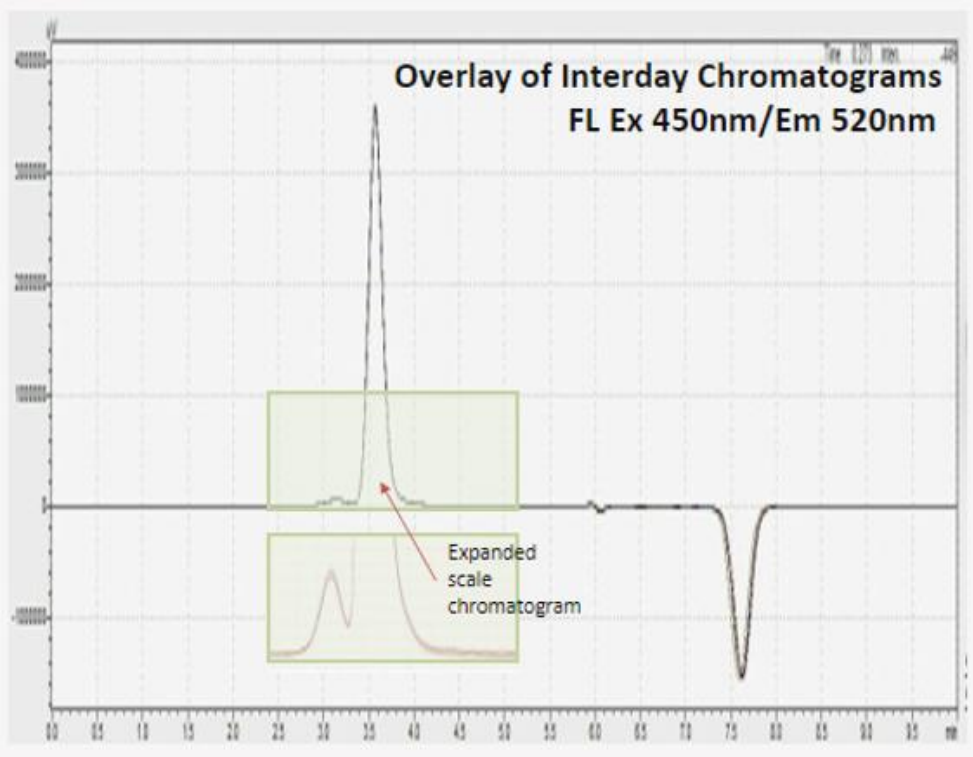

Figure 6. Proteometer-L Chromatograms for 30 Injections over 10 Days

Notes and References:

1. Xu AY, Clark NJ, Pollastrini J, Espinoza M, Kim HJ, Kanapuram S, Kerwin B, Treuheit MJ, Krueger S, McAuley A, Curtis JE. Effects of Monovalent Salt on Protein-Protein Interactions of Dilute and Concentrated Monoclonal Antibody Formulations. Antibodies (Basel). 2022 Mar 31;11(2):24. doi: 10.3390/antib11020024. PMID: 35466277; PMCID: PMC9036246.

2. Patent pending: "A Multiple Affinity Selector Assay System For Critical Structure Attribute Analysis Of Proteins", F. Regnier, et. al.