Quality Assessment of Aglycosylated Therapeutic Monoclonal Antibodies With Proteometer-L Kit

NOVILYTIC

BACKGROUND AND INTRODUCTION

Preventing the conserved glycan modification at Asn-297 (N297) of therapeutic monoclonal antibodies (mAbs) by mutational replacement of Asn with an amino acid such as Ala results in an aglycosylated variant. This variant is nearly identical to the native molecule in terms of biological activity, stability, and pharmacokinetics[1].

Aglycosylated mAbs are cheaper to manufacture than traditional glycosylated therapeutic mAbs. The emergence of aglycosylated therapeutic mAbs means that their critical quality attributes such as titer and relative aggregate composition are reliably monitored throughout product development lifetime to ensure safety and efficacy. Novilytic's Proteometer-L Kit utilizes the new Mobile Affinity Sorbent Chromatography (MASC) Luminon technology for fast and reproducible measurement of mAb titer and relative aggregate content directly in clarified fermentation broth (CFB), avoiding costly and time-consuming Protein A purification steps[2]. This application note evaluates the utility of the Proteometer-L Kit to measure titer and relative aggregate composition of aglycosylated Cetuximab (N297 mutant) biosimilar directly in CFB.

PRINCIPLE

The Proteometer-L Kit uses a molecular recognition agent to detect antibody monomers and aggregates through interaction with a conserved region of the molecule. Its technology is applicable to most therapeutic mAbs, including IgG1, IgG2, and IgG4 subclasses[2]. We show here that the Proteometer-L assay can be used to rapidly and directly monitor titer and aggregate content of aglycosylated Cetuximab biosimilar in CFB without the need for Protein A purification.

RESULTS

CFB was collected from an 8-day-old suspension culture of untransformed CHO cells (viable cell count, 98%). Aglycosylated cetuximab biosimilar (research use only) was formulated in the CFB at 0.5 mg/mL. The Proteometer-L Kit was used to analyze the aglycosylated Cetuximab and CFB (Figure 1). The monomer and aggregates of aglycosylated Cetuximab were well resolved and CFB components did not cause any interference in the assay. The total fluorescence area of the aggregate and monomer peaks is used as a measure of mAb titer. The standard curve for mAb quantification in CFB is illustrated in Figure 2. The R^2 value of the trendline in the plot was 0.9998 or greater, indicating superb linearity. The average aggregate content across the test range of 0.5 to 4 µg aglycosylated Cetuximab (n = 10) was 1.08 % ± 0.12 percent (CV 11.1%).

CONCLUSIONS

The capability of this assay to be performed in CFB makes it an extremely versatile tool with excellent sensitivity of as low as 0.5 µg antibody per assay. Assay results may be obtained within 10 minutes of sampling from a bioreactor with minimal handling.

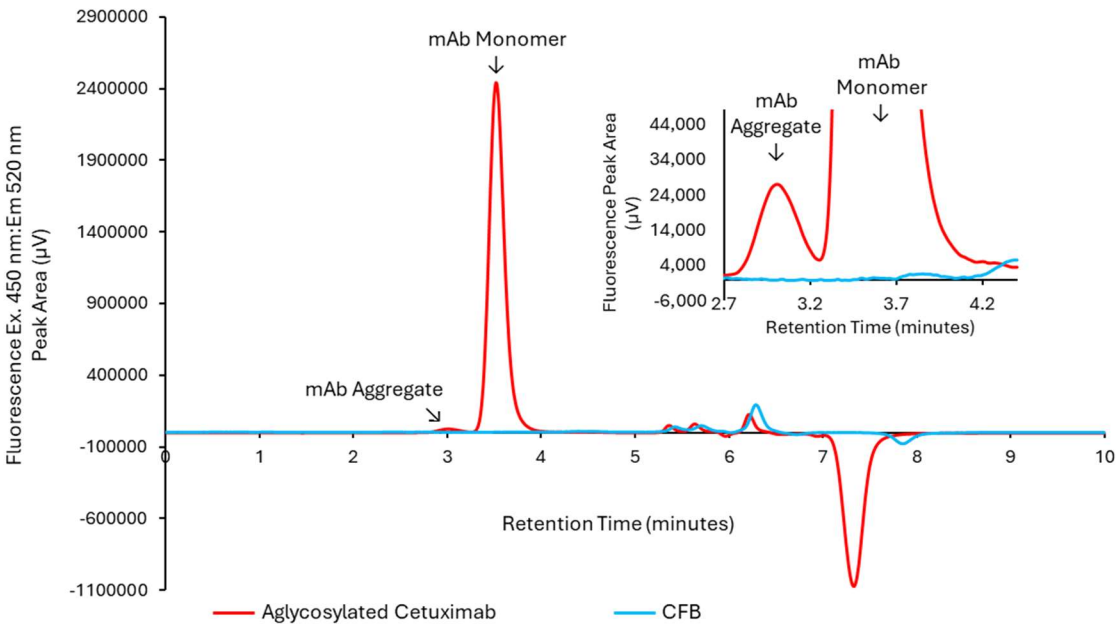

Figure 1. Overlay of chromatograms obtained by Proteometer-L analysis of aggregate and monomer components of aglycosylated cetuximab (4 µg in 8 µL CFB), shown in red and CFB (8 µL), shown in blue. Inset shows magnification of a portion of the overlay between 2.7 min and 4.4 minutes.

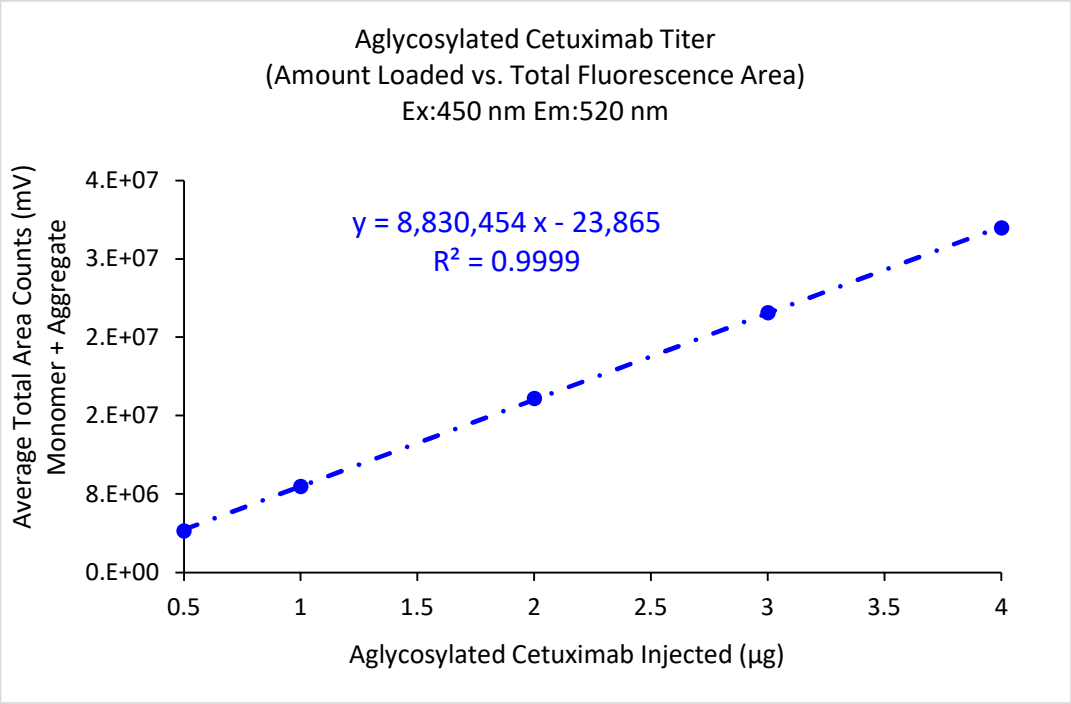

Figure 2. The standard curve for aglycosylated cetuximab in CFB with the Proteometer-L assay. Sample load ranged from 0.5 to 4 µg of mAb in CFB.

REFERENCES

1. Ju MS, Jung, S.T. Aglycosylated full-length IgG antibodies: steps toward next-generation immunotherapeutics. *Current opinion in biotechnology*, 30, 128-139 (2014). https://doi.org/10.1016/j.copbio.2014.06.013
2. Narsimhan ML, Kim J, Morris NA, Bower MA, Gunawardena HP, Bowen E, Regnier FE. Mobile Affinity Selection Chromatography Analysis of Therapeutic Monoclonal Antibodies. Analytical Chemistry. 2023 Oct 26;95(44):16115-22. doi: 10.1021/acs.analchem.3c02180.