Analysis of Denosumab, an IgG2 Subclass mAb, with Proteometer-L Assay

NOVILYTIC

BACKGROUND AND INTRODUCTION

IgG2 monoclonal antibodies (mAbs) are produced in response to bacterial polysaccharide antigens and are associated with protection against respiratory infections[1]. They have a shorter and more highly disulfide-linked hinge region connecting their Fab and Fc domains, which sets them apart from IgG1 and IgG4 subclass mAbs. Relative to IgG1 and IgG4 mAbs, fewer therapeutic mAbs of the IgG2 subclass have been approved for use. However, since 2017, there has been an increase in the number of IgG2 mAbs approved and under investigation, particularly for respiratory and inflammatory disorders[2-5].

We demonstrate here the utility of the Proteometer-L Kit for rapid analysis of titer and relative aggregate content of Denosumab, a mAb of the IgG2 subclass[2], in clarified fermentation broth (CFB) without prior purification by Protein A. Denosumab was approved for use by the Food and Drug Administration in 2010 to treat bone loss, making it the second mAb of the IgG2 subclass to be approved. Denosumab generates approximately $4.4 billion annually and its revenue is expected to increase with an aging population's need for bone loss treatment. It will go off-patent in 2025, and at least 11 companies are fast-tracking approval of biosimilars for commercialization by that time[6]. Rapid analyses for titer and relative aggregate content by the Proteometer-L Kit can expedite early product and process development, both for the newer IgG2 subclass mAbs and biosimilars of off-patent IgG2 mAbs[7].

PRINCIPLE

Monitoring changes in mAb concentration (titer) and aggregate content during the development and manufacture of a mAb is critical, as these can indicate changes in quality attributes of the molecule that can result in lower efficacy and/or higher toxicity of the drug. The Proteometer-L Kit employs Novilytic's patented MASC™ technology for rapid analysis of titer and relative aggregate content of human or humanized mAbs in the presence of matrix impurities. The enabling feature of this technology is a soluble, low molecular weight, fluorescent affinity selector that binds specifically to the Fc domain of IgGs. Only fluorescently coded IgGs are detected while impurities remain invisible.

RESULTS

Denosumab biosimilar (research grade) was formulated at a concentration of 1 mg/mL in clarified fermentation broth (CFB) and injected in varying amounts onto an HPLC system equipped with a fluorescence detector. The Proteometer-L Kit was utilized for the system setup. The peak area responses from triplicate injections, performed on three different days (n = 9) were plotted against the amount (µg) of Denosumab injected. Excellent linearity was observed from the range of 0.5 to 16 µg of mAb injected as shown in Figure 1 (R^2 = 0.9999).

There is no interference from host cell proteins in the CFB sample matrix, and aggregate content is determined without the need for sample cleanup prior to analysis. Data from injections ≥ 4 µg showed an average aggregate (high molecular weight species) to total area percent of 2.15 ± 0.24 for 36 injections with a coefficient of variation (CV) of 11.1%. Repeatability was evaluated by performing 10 consecutive injections of 4 µg Denosumab in CFB in three assay runs, on three different days. The total area, representing the titer for the mAb, showed excellent repeatability with an average area of 42,600,222 ± 378,587 for 30 injections and a CV of 0.89% (Figure 2). The average aggregate content was 1.93% ± 0.06 for 30 injections, with a CV of 2.95% (Figure 3).

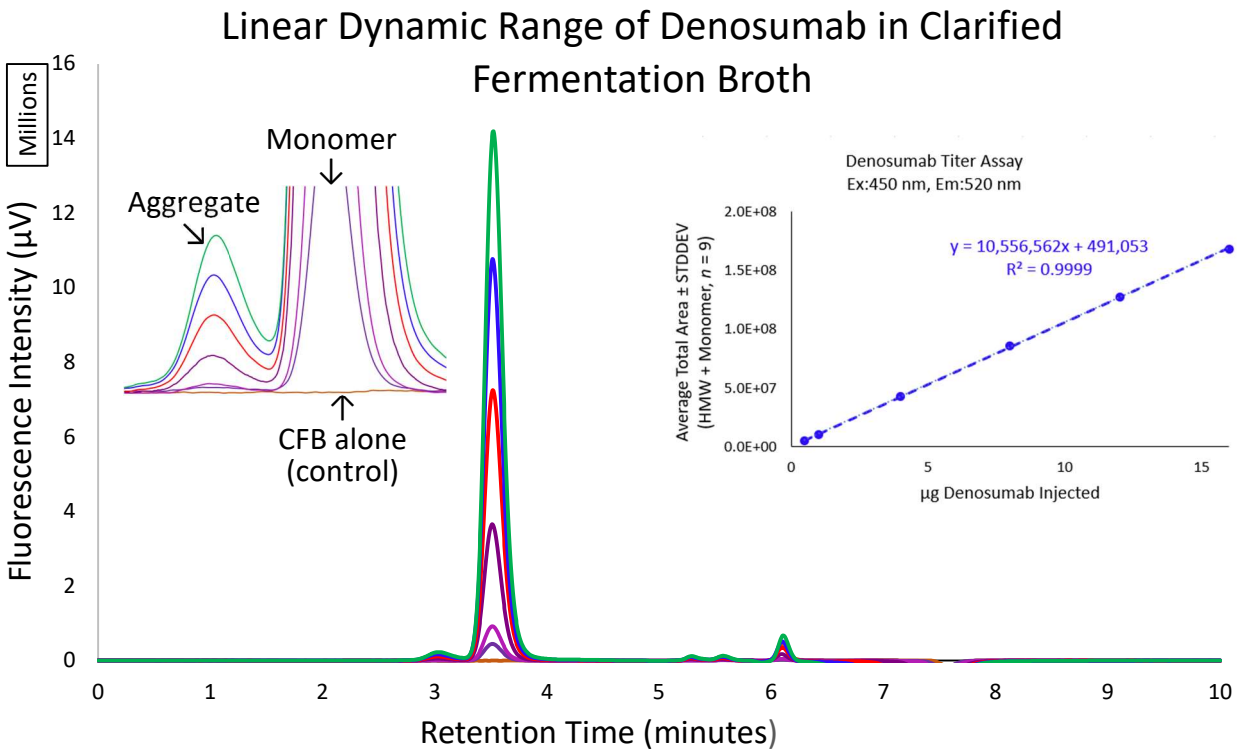

Fig. 1. Linear dynamic range of Denosumab in CFB.

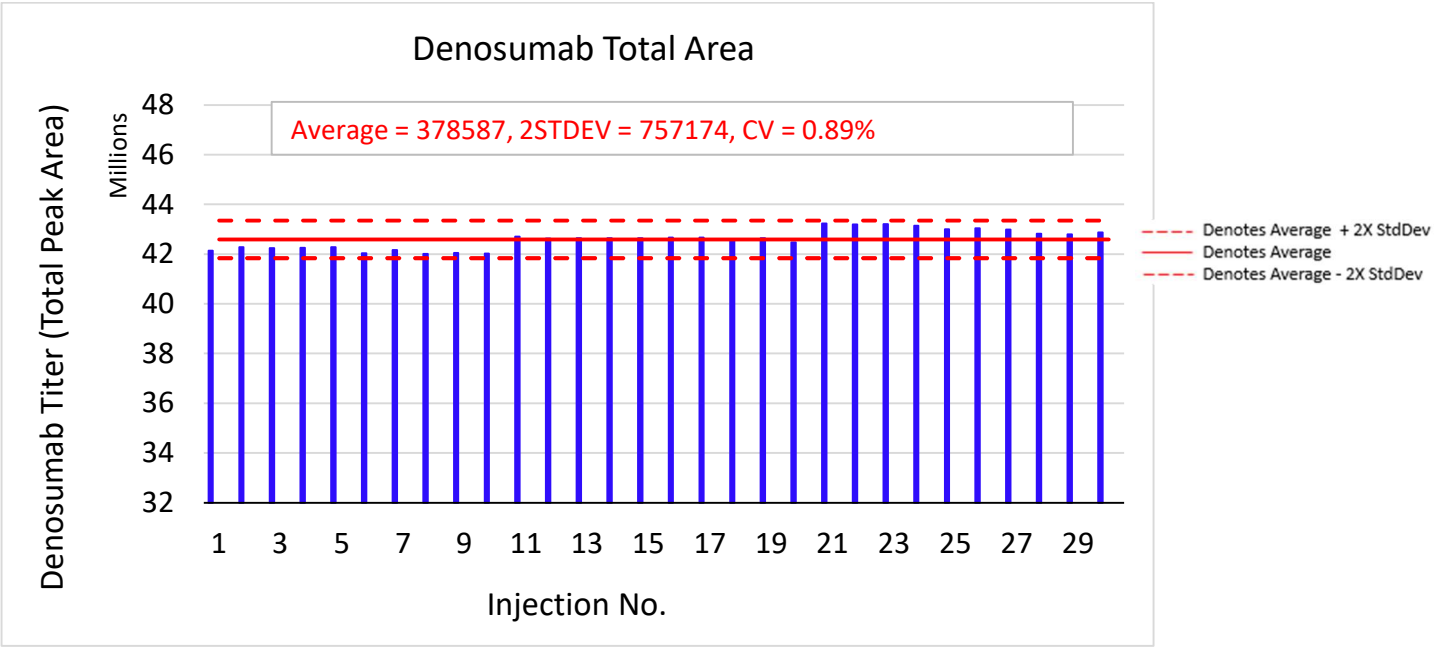

Fig. 2. Repeatability of total peak area observed in Denosumab in CFB.

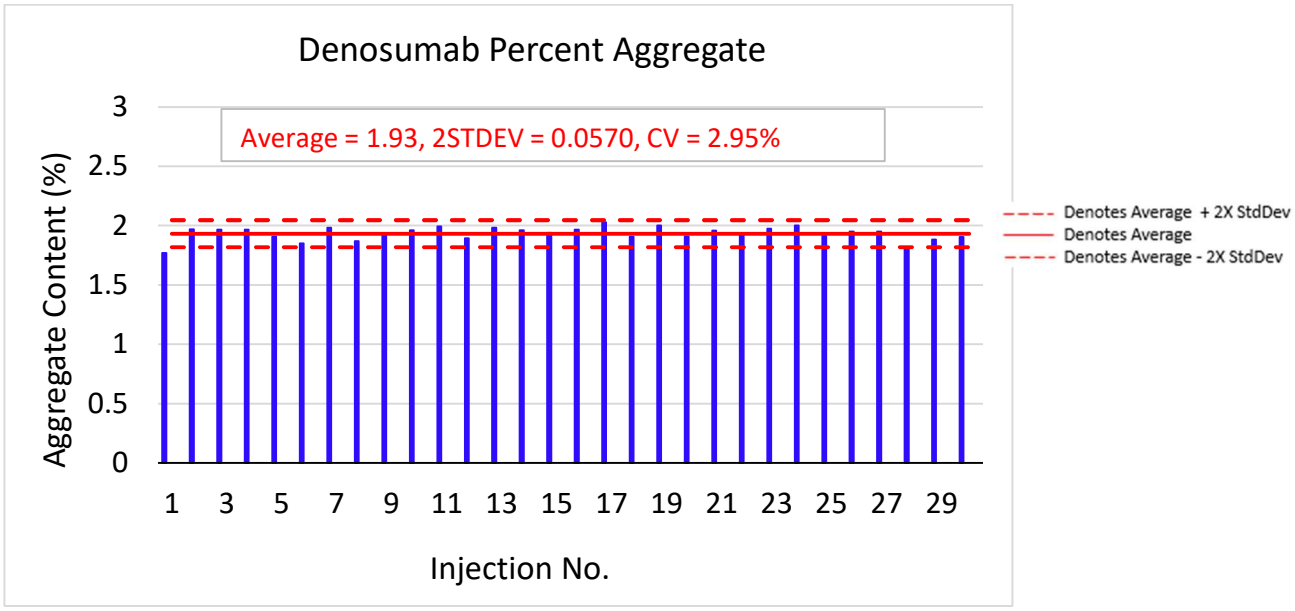

Fig. 3. Repeatability of aggregate content of Denosumab in CFB.

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