Aggregate Analysis of Fc-Fusion Proteins

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Overview

Fc-fusion proteins are Fc homodimers linked to a polypeptide chain. From an analytical standpoint, Fc-fusion proteins present several challenges. Fc-fusion proteins may have much more complex glycosylation, including deviations from the biantennary fucosylated glycans associated with monoclonal antibodies, as well as O-glycans. As glycosylation directly impacts protein conformation or folding, analytical LC separations may be affected as well. Indeed, even with non-adsorptive separation modalities such as size exclusion chromatography (SEC), separation may require extensive method development for optimization to ensure a robust method.

Figure 2 shows an SEC profile for Etanercept, a heavily glycosylated Fc-TNFR conjugate. Because it often has sialylation, non-ideal SEC interactions can be observed. That is, so-called “ion exclusion” of the protein due to repulsion of the negatively charged protein and inherently negative silica. This can cause peak broadening and/or adsorption of the protein, which can prevent the proper quantitation of the protein. As such, the mobile phase used should have sufficient co-solvent (i.e. NaCl or other salt) to minimize this effect. Using a 2X Phosphate Buffered Saline as the mobile phase ensures that any electrostatic interaction is minimized. Thus, percent monomer and aggregate can be easily calculated, using standard integration parameters, with percent monomer being 96.9% by peak area.

This strategy can be implemented for other Fc-Fusion proteins which potentially can present similar issues. **Figure 3** shows a SEC profile for Aflibercept, an Fc-Fusion protein conjugated to VEGF receptor. Although not as complex as Etanercept, Aflibercept being a Fc-Fusion protein often has physicochemical properties that are not as predictable as a canonical IgG1 monoclonal antibody.

LC Conditions

Column: Biozen™ 3 µm dSEC-2, 200 Å
Dimension: 300 x 7.8 mm
Part No.: 00H-4788-K0
Mobile Phase: 50 mM Sodium Phosphate + 300 mM NaCl, pH 6.8
Flow Rate: 1.15 mL/min
Injection Volume: 10 µL
Temperature: 30 °C
Detection: UV @ 280 nm
Sample: As indicated

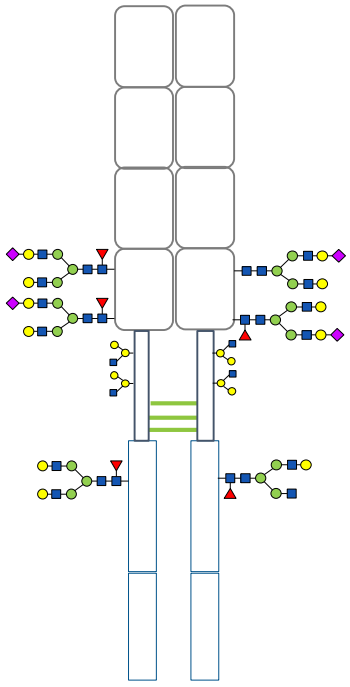


Figure 1. Structure of Etanercept

Figure 2. SEC Profile for Etanercept (25 mg/mL)

SEC Chromatogram for Etanercept, showing good separation of monomer and aggregate. Monomer purity is 96.9% by peak area.

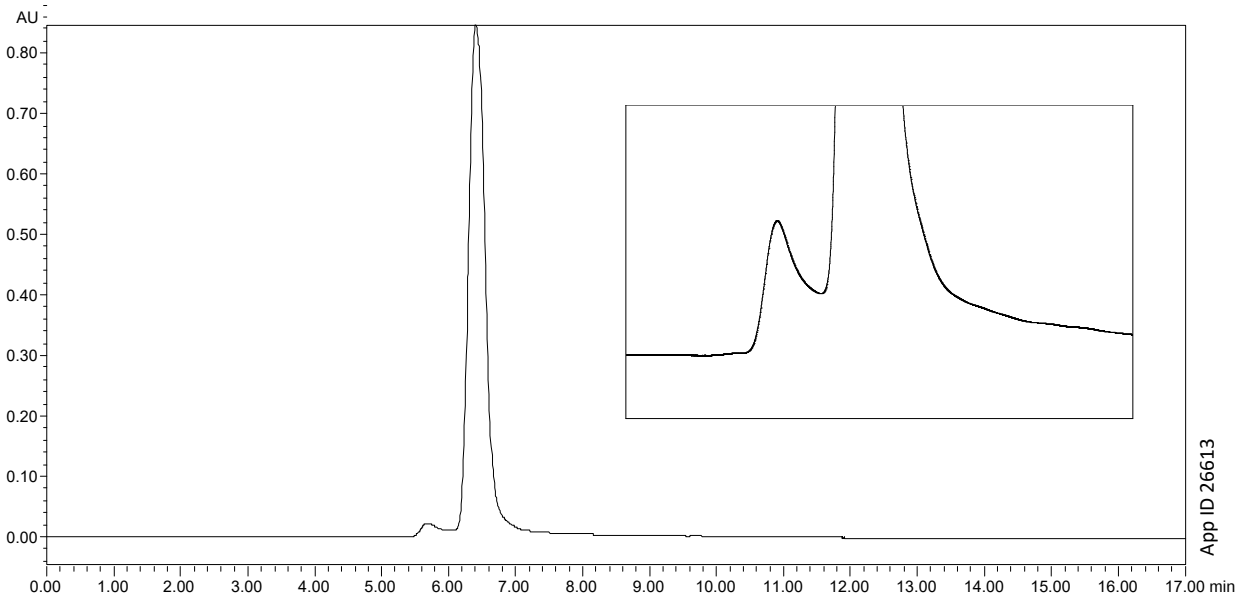
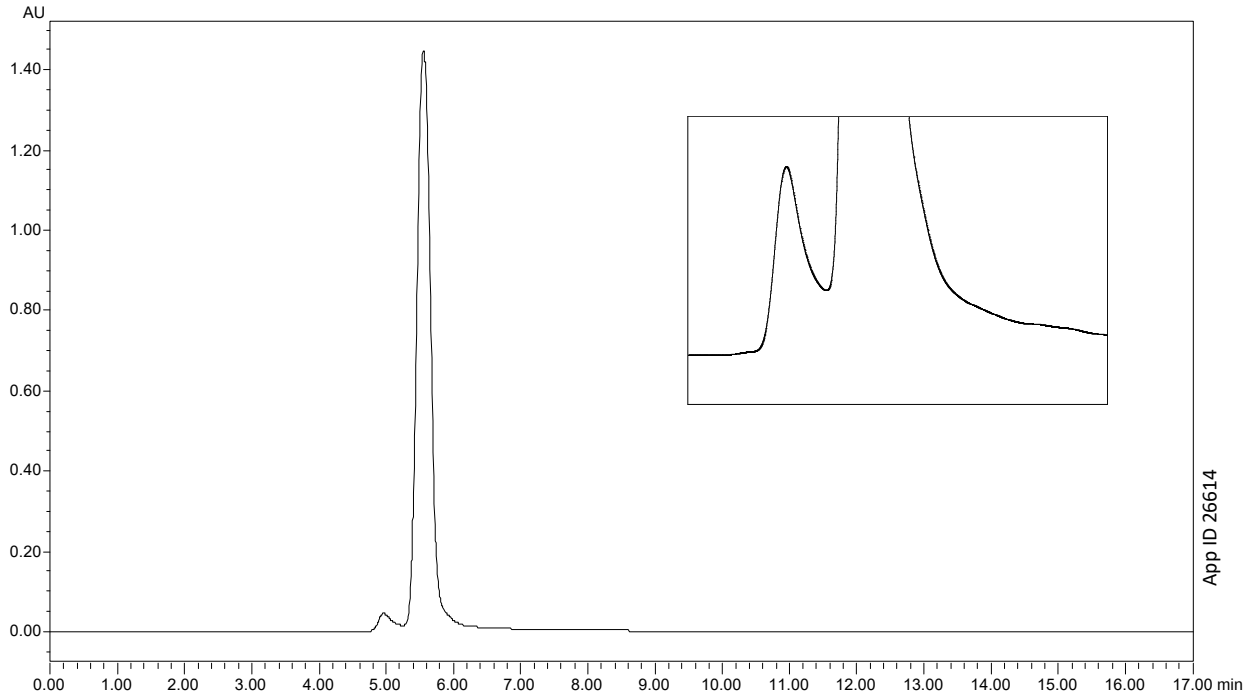


Figure 3. SEC Profile for Aflibercept (25 mg/mL)

SEC Chromatogram for Aflibercept, showing good separation of monomer and aggregate. Monomer purity is 96.8% by peak area.



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