Characterization of Native and PEGylated Fab-arms

TSKgel
APPLICATION NOTE

Introduction

Biopharmaceuticals represent a growing number of therapeutic products on the market and in R&D pipelines at this time. Of the various monoclonal antibody-derivatized products, bispecific monoclonal antibodies (BsAbs) present a unique approach as they are entirely artificially produced through the linkage of the Fab-arms (half-mAbs) of two different monoclonal antibodies (mAbs). BsAbs combine the antigenic properties of two different proteins and incorporate them into a single drug delivery vessel, making them highly applicable to cancer immunotherapy. Similarly, antibody drug conjugates (ADCs) utilize a linker molecule to bind a cytotoxic drug to a monoclonal antibody or Fab-arm to develop a highly targeted, multi-functional drug therapeutic.

BsAbs require careful characterization of the Fab-arms (or half-mAb fragments) to evaluate heterogeneity. Likewise, ADCs must be evaluated for heterogenic impurities to determine the success and extent of drug linkage to the parent antibody, as well as for heterogeneity. The use of size exclusion chromatography (SEC) can allow for the thorough evaluation of such properties of biomolecular therapeutics.

This application note outlines the separation and analysis of a Fab-arm and PEGylated Fab-arm species isolated from a native monoclonal antibody using a TSKgel® SuperSW3000 SEC column.

Experimental Conditions

Column:	TSKgel SuperSW3000, 4 µm, 4.6 mm ID × 30 cm
Mobile phase:	100 mmol/L sodium phosphate/100 mmol/L sodium sulfate, pH 6.7, + 0.05% NaN_3
Flow rate:	0.35 mL/min
Detection:	FLD (λ_{ex}: 280 nm, λ_{em}: 350 nm)
Temperature:	30 °C
Injection vol.:	10 µL

Materials and Methods

- TCEP (Thermo Fisher)
- 30 kDa NHS-PEG (ME-300CS, NOF)
- Sodium cyanoborohydride (Sigma)
- Sodium phosphate (Sigma)

Fab-arm formation*

- 500 mmol/L TCEP was used for protein reduction
- Briefly, 125 µL of protein was mixed with 325 µL SEC mobile phase
- 50 µL TCEP was then added to bring the total volume to 500 µL and the reaction mixture was incubated at 31 ºC for 90 minutes

*method adapted from *Nature Protocols*, 9, 10. 2014, pg. 2457

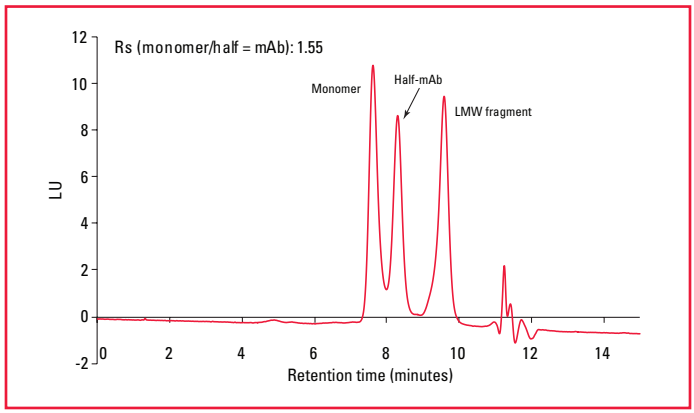
Protein PEGylation

- 100 µL of a 24 g/L solution of 30 kDa PEG in 20 mmol/L $NaCNBH_3$ and 5 mmol/L Na_2HPO_4 was added to 100 µL of protein
- The solution was vortexed and incubated overnight at 8 ºC

Results and Discussion

Figure 1 demonstrates the optimal resolution obtained between a mAb monomer and half-mAb species using a TSKgel SuperSW3000 column (flow rate: 0.35 mL/min). The Rs value of 1.55 indicates complete resolution of the two species. The highly efficient separation of the half-mAb from the mAb monomer and low molecular weight (LMW) fragment allows for a highly purified half-mAb sample.

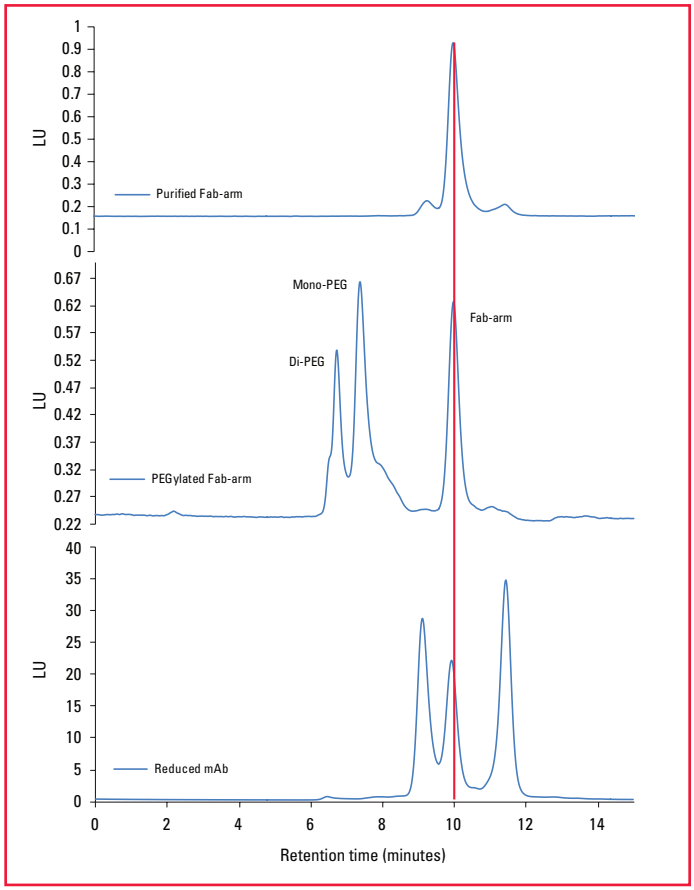
Figure 1. Separation of Fab-arm from mAb Monomer and LMW Fragment



PEGylation is the process of adding polyethylene glycol chains to a compound in order to change the physical properties of such a species. In biopharmaceuticals PEGylation is typically performed in an effort to increase the hydrodynamic radii of a protein-based therapeutic, which typically results in reduced renal clearance, extending the drug's time within the patient. Additionally, PEGylation adds water solubility to hydrophobic drugs due to the attachment of the hydrophilic polyethylene glycol.

Separation of a PEGylated Fab-arm from a non-PEGylated species using a TSKgel SuperSW3000 column is shown in *Figure 2*. The mono- and di-PEGylated species both illustrate significant increases in the hydrodynamic radii, as illustrated by the earlier elution of each species relative to the native Fab-arm. SEC analysis of the reduced mAb using the TSKgel SuperSW3000 column allowed for fraction collection of the Fab-arm.

Figure 2. Separation of PEGylated Fab-arm from Non-PEGylated Species



Conclusion

The use of a TSKgel SuperSW3000 SEC column allows for high resolution separation of native mAbs, and their low molecular weight fragments, including the Fab-arm (half-mAb). The added complexity of PEGylated species can be well characterized by SEC using the TSKgel SuperSW3000 column.

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