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Antibody Drug Conjugate (ADC) Load Characterization and Analysis Using the BioAccord and Nano DSC Systems

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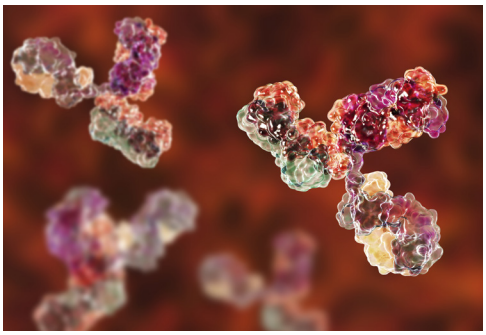

GOAL

Demonstrate the added value of a multi-disciplinary approach to antibody drug conjugate (ADC) analysis by linking the biochemical and biophysical experiment results.

BACKGROUND

ANTIBODY DRUG CONJUGATES

Antibody drug conjugates (ADCs) are a sub-class of biopharmaceuticals that consist of a monoclonal antibody (mAb), linker, and a payload (typically a small molecule drug or toxin). The successful combination of these three components yields a drug that can release its cytotoxic payload with optimal pharmacokinetics into a specific population of targeted cells.[1] Biotherapeutic efficacy is directly related to the drug-to-antibody ratio (DAR) and determination, and monitoring this is essential.

LIQUID CHROMATOGRAPHY (LC), MASS SPECTROMETRY (MS), AND DIFFERENTIAL SCANNING CALORIMETRY ON ADCs

Both biochemical (LC-MS) and biophysical (DSC) tools can be used for ADC characterization and DAR monitoring.

A physicochemical analysis of antibody drug conjugate (ADC) focusing on the drug-to-antibody ratio (DAR).

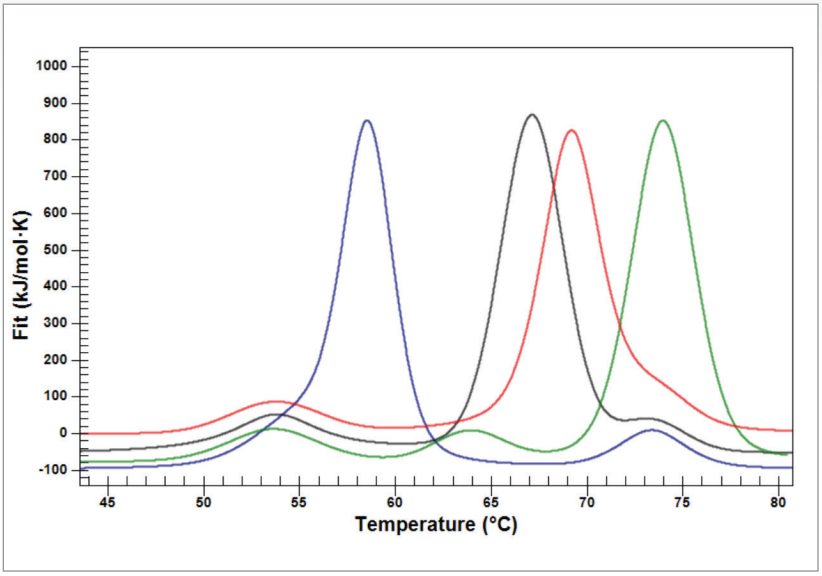

Figure 1. Simulated (green, blue, and red) and real (black) IgG data from DSC analysis. Blue simulated data has two peaks with the CH2 and Fab unfolding overlapping the simulated red data shows overlapping between the CH3 and Fab domain. In the green data, the CH3 domain unfolds prior to the Fab domain.

Separation technologies paired with MS can address size variants (aggregation and fragmentation), drug distribution, DAR, and the amount of free mAb in a solution. The composition and structure of the biotherapeutic dictates which analytical techniques are ideal for the ADC.[2]

The structural perturbation of an mAb, as linkers and drugs are bound, can be assessed through interpretation of a DSC scan; where the stability of a protein is described by its enthalpy (ΔH) and T_m (ΔG relationship). A typical mAb DSC thermogram will have two to three domain unfolding events, two when the CH2 or CH3 overlap with the Fab domain and three when the domains unfold separately[3] (Figure 1). If more than three events are observed, then it is assumed that Fab unfolding is no longer cooperative or there is significant structural heterogeneity.[3,4]

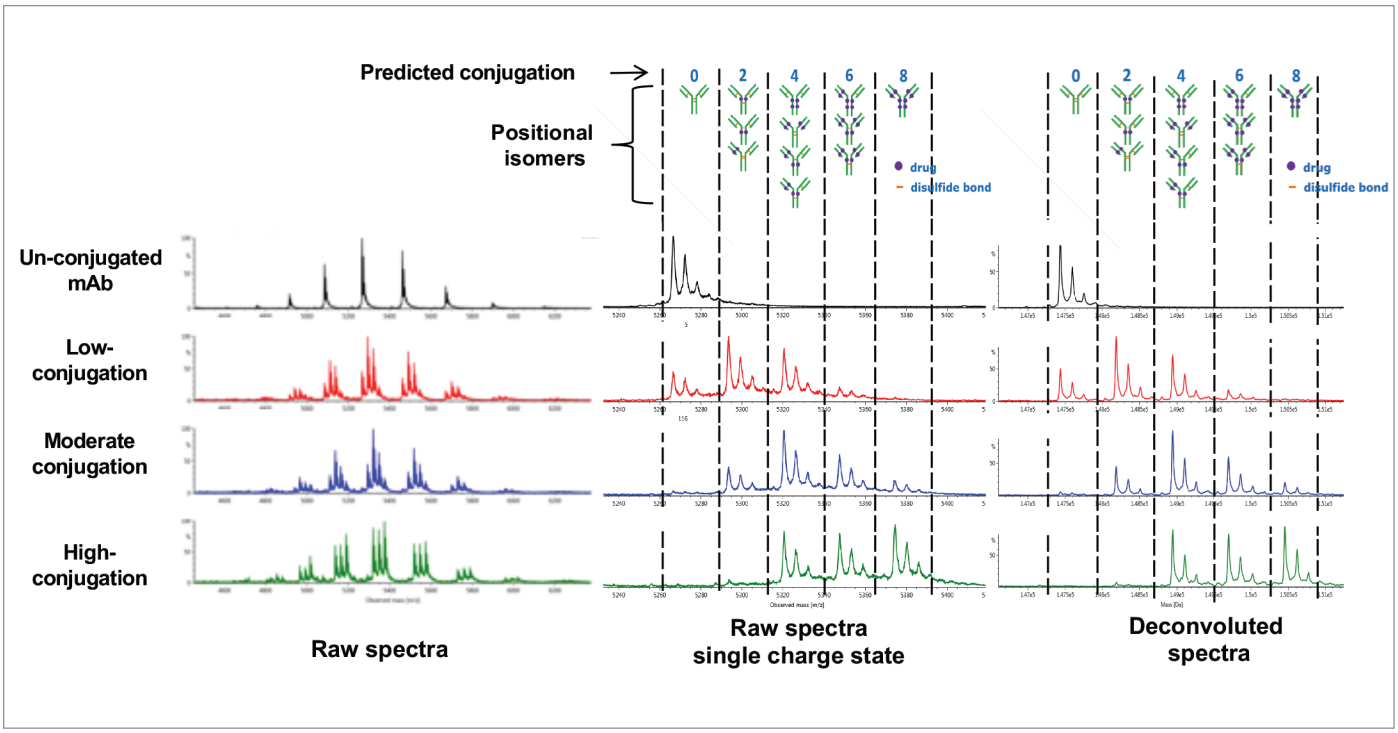

Figure 2. Combined raw spectra from multiple charge state envelop and the combined raw spectra of an mAb with low, moderate, and high DAR of a cysteine-conjugated ADC.[8]

THE SOLUTION

Non-denaturing separations, such as hydrophobic interaction chromatography (HIC) and size exclusion chromatography (SEC), are well-suited for DAR quantification when highly hydrophobic drugs are bound with either lysine or cysteine conjugation sites.[5-8] The Waters™ BioAccord™ System, with the ACQUITY™ UPLC™ I-Class PLUS System with optical detector coupled in-line to an ACQUITY RDa™ Detector, produced DAR and drug distribution data for both lysine and cysteine conjugated ADCs.[8] In a separate study on the same ADC samples, the Nano DSC from TA Instruments® (A Waters Company) was utilized to evaluate ADCs and DAR characterization as well.[9] The cysteine conjugated data collected on the BioAccord System and Nano DSC have been reexamined in the context of identifying points of commonality and synergy.

Data collected on the BioAccord System reveals the frequency and magnitude of the positional isomers, where the highest probability of the drug linkers are on the cysteine residues in the CH2 domain (Figure 2). This observation is consistent

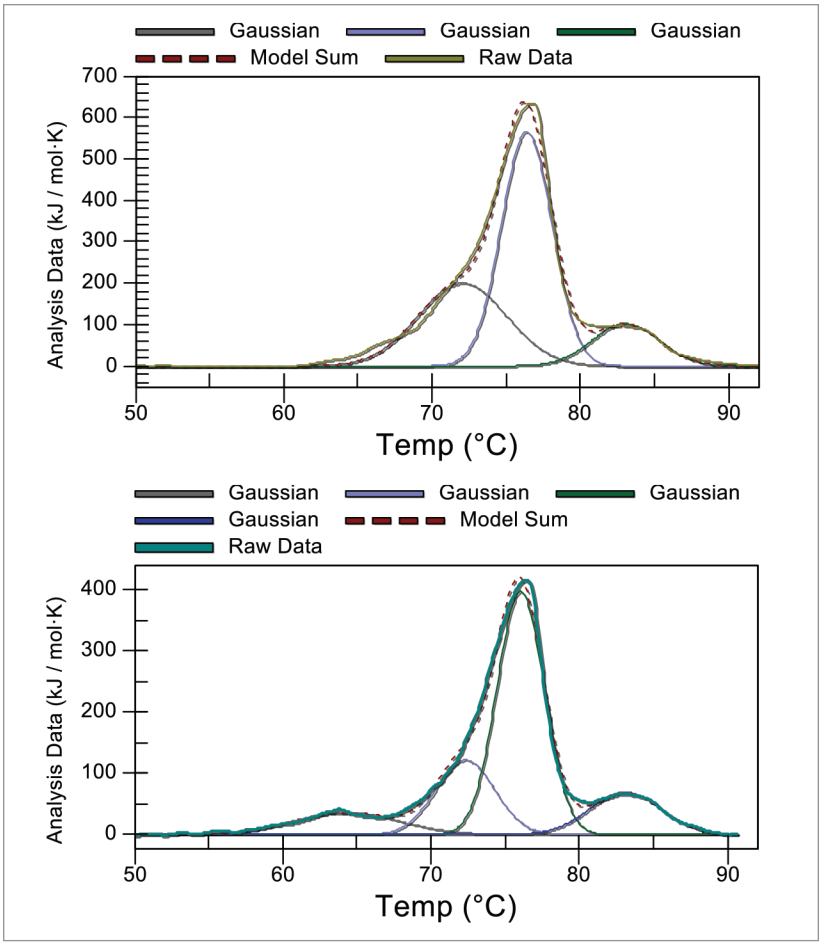

Figure 3. Gaussian-fitted DSC ADC results for native (top) and a high DAR mAb (bottom).[9]

with the DSC thermograms and the appearance of an additional peak, below the native CH2 domain (Figure 3).

Based on the BioAccord System data, this low T_m DSC peak can be assigned as a CH2 domain modified with the linker-drug. This assignment is consistent with the decrease in T_m as the local area around the conjugation has become more hydrophobic, lowering the stability of the region. Furthermore, as the DAR progressed from low to high levels, the new peak grew in enthalpic magnitude, indicating a growing destabilized population.

The decreasing total enthalpy also tells a similar story, with 20% loss at a low DAR, followed by smaller incremental losses of 10% as the average DAR increased. The addition of the hydrophobic molecule still perturbs the system at a higher DAR, but because of the localized addition, the magnitude of the disruption is smaller. This argument can be rationalized by the assignment of the positional isomers from the BioAccord System. The Fab unfolding peak retains its cooperativity based on its symmetry, consistent with the occupational symmetry in the positional isomers in the MS data. Finally, there is no change in CH3 domain stability, which also is supported with the MS data.

SUMMARY

Physicochemical characterization is a strategy that ensures expanded understanding of the biologic system of interest. It takes a molecule through the analytics of biochemical characterization to identify critical quality attributes, into biophysical characterization, with confidence from discoveries supported by orthogonal platforms that confirm assertions made independently. The synergies obtainable from elements of the Waters/TA instrument portfolio, the BioAccord System and Nano DSC, enables a physicochemical strategy that facilitates a rigorous and complementary assessment of the molecule's chemical and physical properties.

References

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