Characterization of Antibody-Drug Conjugates by SEC with Combined Light Scattering, dRI and UV Detection

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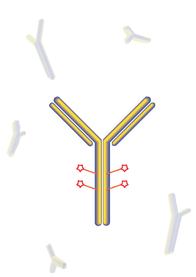
SEC/FFF-MALS • CG-MALS • DLS • MP-PALS

Molar Mass • Size • Interactions • Charge

1. Abstract

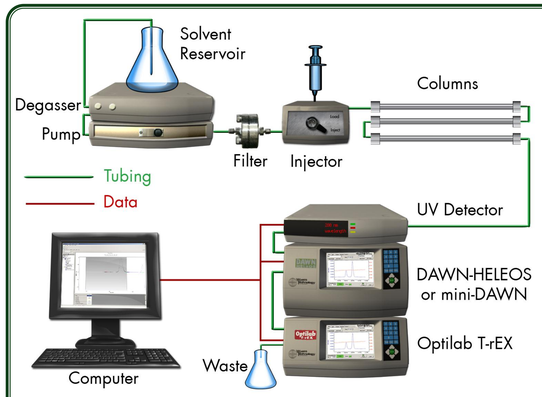
There has been a significant resurgence in the development of antibody-drug conjugates (ADC) as target-directed therapeutic agents for cancer treatment. Among the factors critical to effective ADC design is the Drug Antibody Ratio (DAR). The DAR describes the degree of drug addition which directly impacts both potency and potential toxicity of the therapeutic. Determination of DAR is, therefore, of critical importance in the development of novel ADC formulations. DAR is typically assessed by mass spectrometry (MALDI-TOF or ESI-MS) or UV spectroscopy. Calculations based on UV absorption are often complicated by similarities in extinction coefficients of the antibody and small molecule. Mass spectrometry, though a powerful tool for M_w determination, depends on uniform ionization and recovery between compounds—which is not always the case for ADCs. We present here a method for DAR determination based on SEC-MALS in conjunction with UV absorption and differential refractive index detection.

2. What are Antibody-Drug Conjugates?



Antibody-drug conjugates wed the targeting specificity imparted by antibodies to the toxicity of small molecule chemotherapeutics. These drugs are covalently attached via short linkers to mAbs raised against cellular targets specific to or upregulated on tumor cells. The antibody delivers its deadly payload preferentially to tumor cells, simultaneously improving treatment efficiency while minimizing collateral toxicity and the associated side-effects of traditional chemotherapeutic effects.

3. Setup



- Agilent 1200 HPLC pump
- Agilent 1200 UV detector
- Wyatt HELEOS II MALS detector
- OptiLab T-rEX dRI
- Eluent: PBS (50 mM phosphate, 50 mM NaCl), 0.5 ml/min

- WTC 030S5G and 030S5 guard and analytical columns
- Samples: ADC1, 2 and 3 (varying degrees of conjugation)
- Protein Conjugate Analysis in ASTRA 6

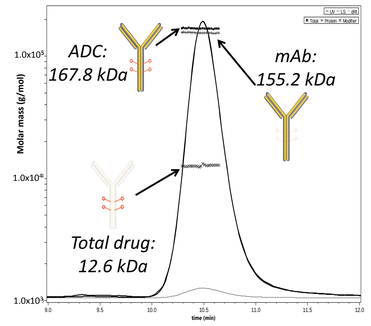
4. How Conjugate Analysis Works

$$\left(\frac{dn}{dc}\right)_{ADC} = \left[\left(\frac{dn}{dc}\right)_{mAb} \cdot X_{mAb}\right] + \left[\left(\frac{dn}{dc}\right)_{drug} \cdot \left(1\text{-}X_{mAb}\right)\right]$$

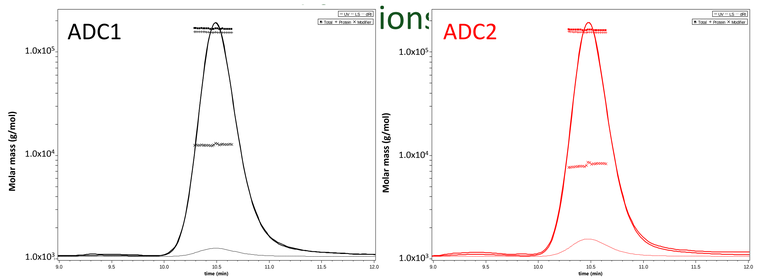
$$\varepsilon_{ADC} = \left[\varepsilon_{mAb} \cdot X_{mAb}\right] + \left[\varepsilon_{drug} \cdot \left(1\text{-}X_{mAb}\right)\right]$$

ASTRA determines concentrations and weight fractions for the mAb (x) and drug (1-x) *independently* based on their unique dn/dc and extinction coefficients. These not only provide assignment of a corrected dn/dc to the complex but also allows determination true molecular weights for the ADC as well as the mAb and total drug sub fractions (right).

Mw vs. Elution Time



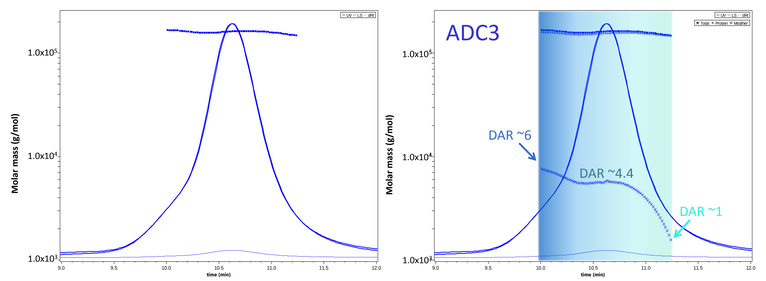
5. Homogenous ions



Two distinct formulations of a mAb alkylated with a small molecule exhibit similar elution profiles. Conjugate analysis reveals the differential modifications. A nominal pendant M_w of 1250 Da was used to calculate the DAR. Horizontal M_w profiles indicate DAR homogeneity.

	M_w (kDa)			DAR
	Complex	Antibody	Drug	
ADC1	167.8 (±1.2%)	155.2 (±1.8%)	12.6	10.1
ADC2	163.7 (±1.2%)	155.6 (±1.2%)	8.1	6.5
ADC3	159.8 (±0.1%)	154.5 (±0.2%)	5.3*	4.2*

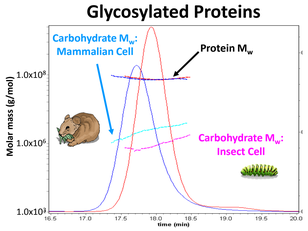
6. Heterogeneous Modifications



Another ADC formulation exhibited a broad and asymmetric elution profile suggesting sample heterogeneity. This was confirmed by conjugate analysis which revealed a polydisperse distribution of species with DAR ranging from ~1 to ~6.

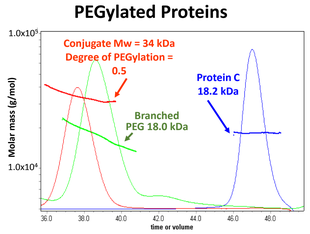
7. Other Applications for Conjugate Analysis

Glycosylated Proteins



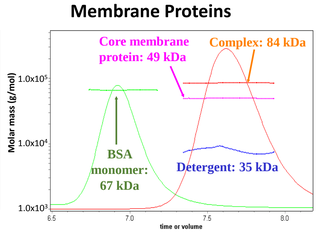
Here a single recombinant protein was observed to exhibit distinct elution profiles whether expressed in mammalian or insect cells, thereby suggesting different molecular weights. Conjugate analysis revealed that, although protein molecular weights are identical, the degree of glycosylation varies between the two expression systems.

PEGylated Proteins



Modification of proteins with polyethylene glycol (PEG) has been shown to increase solubility and serum half-life and reduce toxicity and immunogenicity. As with ADCs characterizing the degree of PEGylation is crucial to development of therapeutics. Here a protein was decorated by a branched PEG molecule. Scattering analysis indicates a degree of PEGylation of 0.5.

Membrane Proteins



Membrane proteins are soluble only in the presence of detergent micelles. Complexation with detergent not only increases M_w but often changes the elution/migration properties of the protein in SEC. Conjugate analysis provides molecular weights for each subcomponent, revealing the amount of detergent bound as well as the oligomeric form of the core membrane protein.

8. Conclusions

- *Protein Conjugate Analysis in ASTRA allows rapid determination of the molecular weights of subcomponents in Antibody Drug Conjugates.*
- *Conjugate analysis is suitable for characterization of Drug-Antibody Ratios in homogenous and heterogeneous ADC populations.*
- *Conjugate analysis is applicable to a wide range of sample types comprising both covalent and non-covalent protein modifications and other bi-component complexes.*