Characterization of Antibody Drug Conjugates using Microfluidic CE-MS

Erin A. Redman, Joshua P. Guerrette, Michael P. Goodwin, J. Scott Mellors
908 Devices Inc., Boston, MA 02210

Overview

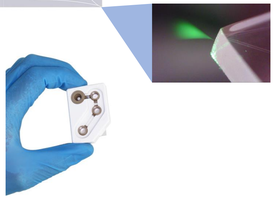
- A microfluidic CE-MS interface was used for the characterization of biotherapeutic monoclonal antibodies and ADC mimics. Multiple levels of analysis were demonstrated to characterize the molecules.
- Charge variants of the intact molecules were separated and analyzed via on-line ESI-MS. After conjugation, the complexity of the IgG increased significantly but DAR species and other variants of the ADC mimics were characterized without additional sample prep.
- Additional middle-up analysis of the IgG and ADC mimics revealed further information about the extent of conjugation and the localization of the modified residues.
- All types of analyses were complete in less than 5 minutes indicating that the microfluidic interface is a simple, rapid means of characterizing complex mAb based therapeutics.

Introduction

Capillary electrophoresis (CE) has been demonstrated to be effective for analyzing complex biological samples, such as monoclonal antibodies (mAbs) and other protein based therapies. When interfaced with mass spectrometry (MS) analysis the integrated techniques present a powerful platform for characterizing biomolecules. Utilizing microfluidic technology to perform CE-MS analysis enables much faster and more efficient separations than can be achieved using capillary tubes. The work described here utilizes a microchip CE-MS interface for the characterization of IgGs and antibody drug conjugate (ADC) mimics.

Methods

ZipChips™ utilize microfluidic technology to harness the inherent speed and efficiency of zone electrophoresis separations. The device design incorporates an injection cross, serpentine separation channel, and an integrated ESI emitter where electrospray is generated directly off the corner of the device. Highly uniform and stable surface coatings suppress the electroosmotic flow and yield highly efficient separations.



Sample prep. ADC mimics were generated using commercially available antibody dye conjugation kits (Thermo Scientific). For analysis, intact IgGs and ADC mimics were diluted to 0.5 mg/mL with LC/MS water. No further sample prep was necessary.

After dilution to 0.5 mg/mL, reduction of the intact molecules was performed with 10 mM DTT in 50 mM ammonium bicarbonate buffer (pH~7). Samples were incubated at 37C for 30 minutes.

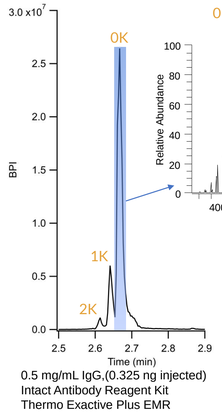
IgGs and ADC mimics were digested with the IdeS protease (Fabricator, Genovis) according to manufacturer specifications in 50 mM ammonium bicarbonate buffer (pH~6.5). Digested molecules were then reduced with 10 mM DTT and diluted to 0.5 mg/mL for analysis.

Analysis. ZipChipHR chips were used for all analyses. Intact analysis was performed using the Intact Antibody Analysis Kit (908 Devices, Inc.). Middle-up analysis was performed using a mixture of methanol/water/formic acid. All separations were performed at 500 V/cm.

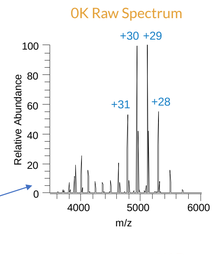
Data Collection and Processing. An Exactive Plus EMR was used for the intact mAb and ADC characterization. Middle-up analysis of the mAbs and ADC mimics was performed on a Thermo LTQ-XL. Biopharmafinder 2.0 was used for data processing. Images were generated using Igor Pro 7 (WaveMetrics, Inc.).

Unconjugated IgG

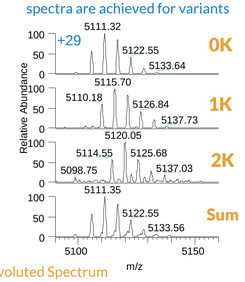
This analysis technique is capable of separating charge variants of intact monoclonal antibodies while also yielding high quality MS data in real time. Interfacing the chip separation with online MS analysis enhances the capabilities of the MS and enables rapid and thorough characterization of biotherapeutic products.

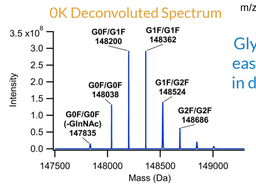


0.5 mg/mL IgG,(0.325 ng injected)
Intact Antibody Reagent Kit
Thermo Exactive Plus EMR



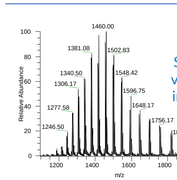
With electrophoretic separation cleaner spectra are achieved for variants



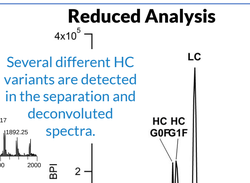


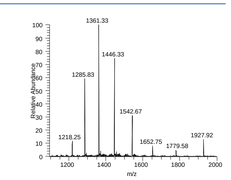
Glycoforms are easily identified in deconvoluted spectra

Reduced Analysis

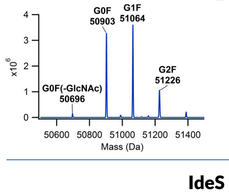


Several different HC variants are detected in the separation and deconvoluted spectra.

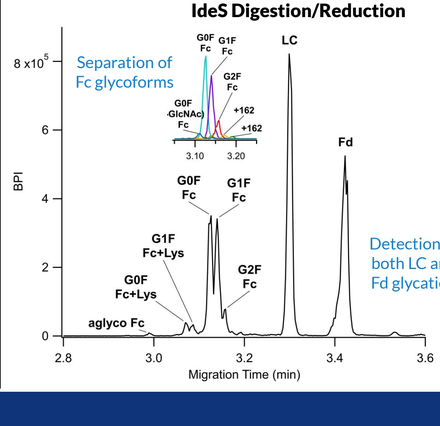


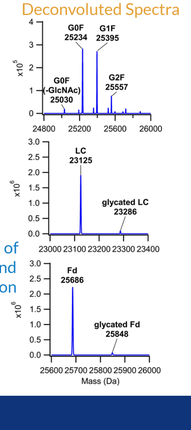


Low levels of light chain glycation are detected.



IdeS Digestion/Reduction

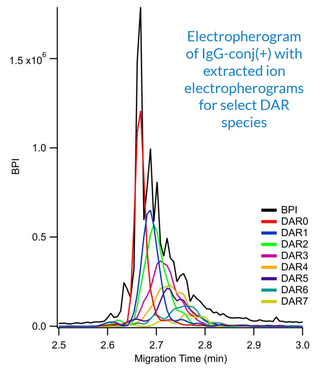




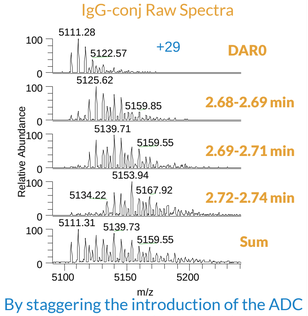
Detection of both LC and Fd glycation

IgG Conjugated with Cationic Drug Mimic

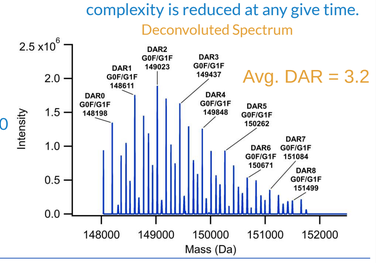
ADC mimics were made using commercially available NHS-Dye reagents to create linkages at solvent accessible lysine residues. The non-specific nature and thoroughness of the conjugation reaction resulted in very complex mimics.



0.5 mg/mL IgG-conj
Intact Antibody Reagent Kit
Thermo Exactive Plus EMR

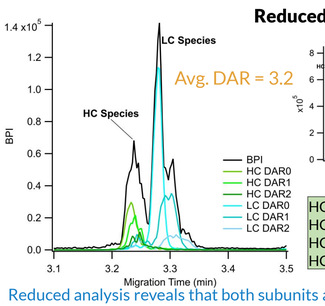


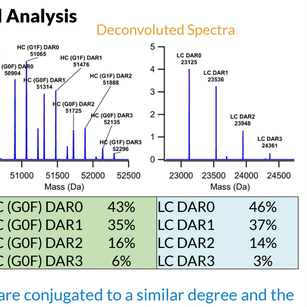
By staggering the introduction of the ADC species into the MS via the electrophoretic separation, the spectral complexity is reduced at any give time.



Utilizing the sliding window algorithm of BioPharmaFinder 2.0 further takes advantage of this separation property to assist in characterizing complex molecules.

Reduced Analysis

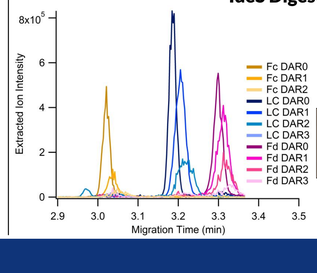




HC (G0F) DAR0	43%	LC DAR0	46%
HC (G0F) DAR1	35%	LC DAR1	37%
HC (G0F) DAR2	16%	LC DAR2	14%
HC (G0F) DAR3	6%	LC DAR3	3%

Reduced analysis reveals that both subunits are conjugated to a similar degree and the calculated Avg DAR agrees with the results from intact analysis of the ADC mimic.

IdeS Digestion/Reduction

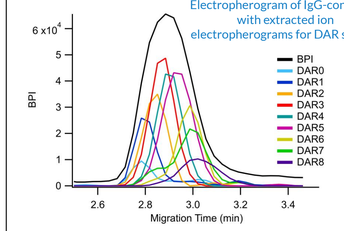


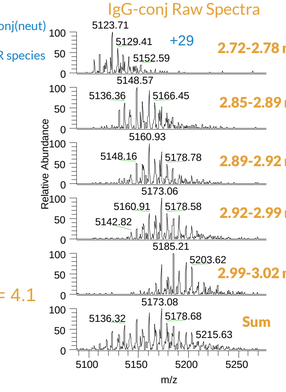
After digestion and reduction an additional HC DAR species was detected as Fd DAR4. Further it is apparent that a majority of the HC conjugation occurs on the Fd portion of the chain.

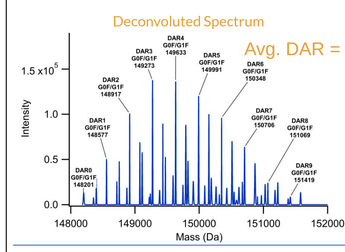
Fc (G0F) DAR0	67%	LC DAR0	34%	Fd DAR0	34%
Fc (G0F) DAR1	24%	LC DAR1	41%	Fd DAR1	37%
Fc (G0F) DAR2	9%	LC DAR2	20%	Fd DAR2	20%
		LC DAR3	5%	Fd DAR3	7%
				Fd DAR4	2%

IgG Conjugated with Neutral Drug Mimic

Conjugation with the neutral drug mimic had a greater impact on the electrophoretic mobility of the IgG molecule causing the DAR species to elute over a greater timeframe.

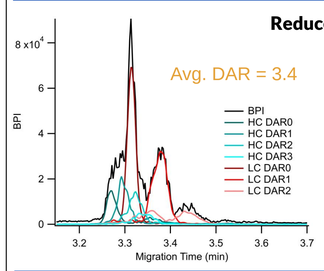






Conjugation with the neutral drug mimic resulted in more extensive conjugation and a higher average DAR.

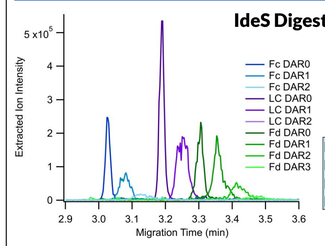
Reduced Analysis



The average DAR calculated from the reduced analysis differs from the value calculated from the intact analysis. This is potentially due to solubility or mobility issues with highly conjugated subunits. Further experimentation is necessary to determine the cause.

HC (G0F) DAR0	30%	LC DAR0	47%
HC (G0F) DAR1	40%	LC DAR1	40%
HC (G0F) DAR2	24%	LC DAR2	12%
HC (G0F) DAR3	5%	LC DAR3	1%
HC (G0F) $DAR_{n>3}$	1%		

IdeS Digestion/Reduction



Conjugation with the neutral drug mimic induced a much greater change in migration time due to the reduction in net charge of the ADC subunits.

Fc (G0F) DAR0	33%	LC DAR0	39%	Fd DAR0	34%
Fc (G0F) DAR1	38%	LC DAR1	42%	Fd DAR1	38%
Fc (G0F) DAR2	22%	LC DAR2	17%	Fd DAR2	21%
Fc (G0F) DAR3	6%	LC DAR3	2%	Fd DAR3	6%
				Fd DAR4	1%

Conclusions

The microfluidic interface is an effective tool for characterizing biotherapeutic proteins. The interface can be used to generate high efficiency separations of intact mAb variants with online MS analysis for thorough characterization of mAb and complex ADC samples. Reduced analysis and digestion with IdeS protease was demonstrated for obtaining chain specific modifications and additional detail about conjugation. The short analysis times for both intact analysis and middle-up analysis make this technique amenable to implementation as a routine method for characterizing molecules and monitoring key product attributes.