Capillary Electrophoresis — Mass Spectrometry for Intact Mass Analysis of Antibodies and ADCs

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ABSTRACT
Correct identification of heterogeneity of monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs) is an essential analytical tool in the biopharmaceutical industry. High-resolution accurate mass spectrometry (HRAMS) has established itself as a powerful tool for the characterization of such compounds; however some proteoforms might not be observed due to potential sample complexity. Here, we used the ZipChip™ system from 908 Devices, to separate and detect the proteoforms of the antibody-drug conjugate NIST mAb-ADC. The preparation of the mAb-ADC has been reported elsewhere. The ZipChip™ system from 908 Devices separates and detects the proteoforms of the NIST mAb-ADC. The mAb-ADC is separated and detected as multiple proteoforms. By using the BioPharma Finder 2.0, we could identify the proteoforms with the help of advanced mass spectrometry. The results show that the BioPharma Finder 2.0 is a powerful tool for the identification of proteoforms in complex biological samples.

INTRODUCTION
The main goal of this work was to develop a method to characterize heterogeneous mAbs and ADCs in a top-down approach. This was achieved by combining the powerful combination of efficient separation by capillary electrophoresis (CE) and HRAMS together with data analysis software. The separation of proteoforms charge variants is quite a challenge using chromatography, but CE provides an ideal separation technique for this problem. Microfluidic CE is advantageous for the mAb analysis given its good separation efficiency and short migration times because of high field strength and short separation channel length. The ZipChip™ mass spectrometer family is a good option for this application, and the Q Exactive HF MS with BioPharma option, with its extended m/z 8000 range, is particularly well-suited to the analysis of very large biomolecules. The BioPharma Finder software package is specifically designed to characterize complex proteins using dedicated deconvolution of mass spectra for individual time windows in combination with a thorough comparison to a reference database with all variants. In this study, the combination of microfluidic CE, separation, MS detection, and data analysis is presented to solve one of the most challenging workflows in the biopharmaceutical industry.

MATERIALS AND METHODS
The NIST reference material (NIST mAb standard REF NIST®) used in this study has a published sequence including post-translational modifications (PTMs). It is a recombinant humanized IgG1 antibody with any process-related impurities removed through various purification steps. The heavy chain of this mAb is known to have a high abundance of PTMs such as N-terminal pyroglutamation, c-terminal/lysine clipping, and glycosylation. 14gplu, 15gplu, 16gplu, and 17gplu, were used as sample dilution with deionized water to 0.5gplu. Prior to injection, no desalting procedure is necessary for this series of CEMS experiments. The ZipChip™ HR chip was printed with background electrolyte solution (BGE) consisting of 0.2% acetic acid and 10% IPA (pH=3.7). Once a stable electrospray was observed, 14gplu, 15gplu, and 16gplu were pipetted into the sample reservoir. Pressure injection with 2 psi for 4s was used for sample loading, introducing 3.6gplu, corresponding to 0.2ng of mAb into the electrophoresis channel. 23mV was applied for CE separation within 22 cm-length of channel, and 324v was used for sample injection. Each run was performed in less than 3 minutes. The NIST mAb standard was labeled using click chemistry with a negatively charged fluoresceine derivative or biotin, which were attached on both N- and C- termini. The BioPharma Finder 2.0 was used to identify the proteoforms of the NIST mAb-ADC. The BioPharma Finder software package is specifically designed to characterize complex proteins using dedicated deconvolution of mass spectra for individual time windows in combination with a thorough comparison to a reference database with all variants. In this study, the combination of microfluidic CE, separation, MS detection, and data analysis is presented to solve one of the most challenging workflows in the biopharmaceutical industry.

RESULTS
Figure 2. A) Schematic of high-resolution (HR) microfluidic CE chip with a 22 cm channel and integrated nano-ESI emitter. The nano-ESI spray can be monitored with the integrated red laser and camera. B) Schematic of Q Exactive Plus and BioPharma option.

CONCLUSIONS
- The BioPharma Finder software package is specifically designed to characterize complex proteins using dedicated deconvolution of mass spectra for individual time windows in combination with a thorough comparison to a reference database with all variants. In this study, the combination of microfluidic CE, separation, MS detection, and data analysis is presented to solve one of the most challenging workflows in the biopharmaceutical industry.
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REFERENCES

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