Capillary Electrophoresis – Mass Spectrometry for Intact Mass Analysis of Antibodies and Antibody-Drug-Conjugates

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ABSTRACT

Capillary electrophoresis – mass spectrometry (CE-MS) is an essential analytical tool in the biopharmaceutical industry. High-resolution mass spectrometry (HRMS) has established itself as a powerful tool for the characterization of such complex molecules; however, some pretreatment steps might not be observed due to potential electrophoretic interference. In this work, we studied a ZipChip™ system from 908 Devices to determine the feasibility of HRMS analysis of complex molecules. A ZipChip™ HR microfluidic chip was primed with background electrolyte solution (BGE) consisting of 0.2% acetic acid and 10% IPA (pH=3.17). Once a stable desolvation flow was achieved, the system was ready for sample loading. Pressure injection with 2 psi for 4s was used for sample loading, followed by application of 200V/cm electric field strength for 2 minutes. The NIST reference material (NIST mAb standard RM 8671) used in this study was diluted with deionized water to 0.5 mg/mL. The NIST mAb and its composition were identified via CEMS, among them 5 glycosylated variants including 4 G0F/G0F and 1 G1F/G1F. This identified mAb also had charge variants, which were successfully separated and identified with the correct MS with CID. The identification of different glycoforms of the NIST mAb was performed using the BioPharma Finder™ 2.0 software package, accurate intact mass for at least 15 glyco-forms was determined with a tolerance of 30 ppm.

INTRODUCTION

The main goal of this work was to develop a method to characterize antibody drug conjugates (ADC) using capillary electrophoresis (CE) and mass spectrometry (MS). CE is particularly well-suited to the analysis of complex biological systems due to its high resolving power and ability to separate charged proteins. CE-MS has become an essential tool in the biopharmaceutical industry for the characterization of complex molecules, such as antibodies and antibody-drug conjugates (ADCs). The separation of complex mixtures is critical for the identification and characterization of ADCs, including the detection of impurities and post-modifications.

MATERIALS AND METHODS

The NIST reference material (NIST mAb standard RM 8671) used in this study has been published previously in an international standardization (PNST) as a recombinant humanized IgG1x antibody with six 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-linked glycosylation, cysteine disulfide bonds, and phosphorylation. The CE conditions used in this study were sufficient to resolve the NIST mAb and its composition. The separation procedure is briefly described below: the ZipChip™ HR microfluidic chip was primed with background electrolyte solution (BGE) consisting of 0.2% acetic acid and 10% IPA (pH=3.17). Once a stable desolvation flow was achieved, the system was ready for sample loading. Pressure injection with 2 psi for 4s was used for sample loading, followed by application of 200V/cm electric field strength for 2 minutes. The NIST reference material (NIST mAb standard RM 8671) used in this study was diluted with deionized water to 0.5 mg/mL. The NIST mAb and its composition were identified via CEMS, among them 5 glycosylated variants including 4 G0F/G0F and 1 G1F/G1F. This identified mAb also had charge variants, which were successfully separated and identified with the correct MS with CID. The identification of different glycoforms of the NIST mAb was performed using the BioPharma Finder™ 2.0 software package, accurate intact mass for at least 15 glyco-forms was determined with a tolerance of 30 ppm.

CONCLUSIONS

• NISTmAb and NISTmAb-derived ADC samples with charge variants were separated and characterized using microfluidic glass chips in a CEMS sample complexity mass spectrometry. We used the ZipChip™ system from 908 Devices to determine the feasibility of HRMS analysis of complex molecules. A ZipChip™ HR microfluidic chip was primed with background electrolyte solution (BGE) consisting of 0.2% acetic acid and 10% IPA (pH=3.17). Once a stable desolvation flow was achieved, the system was ready for sample loading. Pressure injection with 2 psi for 4s was used for sample loading, followed by application of 200V/cm electric field strength for 2 minutes. The NIST reference material (NIST mAb standard RM 8671) used in this study was diluted with deionized water to 0.5 mg/mL. The NIST mAb and its composition were identified via CEMS, among them 5 glycosylated variants including 4 G0F/G0F and 1 G1F/G1F. This identified mAb also had charge variants, which were successfully separated and identified with the correct MS with CID. The identification of different glycoforms of the NIST mAb was performed using the BioPharma Finder™ 2.0 software package, accurate intact mass for at least 15 glyco-forms was determined with a tolerance of 30 ppm.

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TRADEMARKS/LICENSING

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References


Figure 1. A) Schematic of high-resolution (HR) microfluidic CE chip with a 22 cm channel and integrated reservoir. Pressure injection with 2 psi for 4s was used for sample loading, followed by application of 200V/cm electric field strength for 2 minutes. The NIST reference material (NIST mAb standard RM 8671) used in this study was diluted with deionized water to 0.5 mg/mL. The NIST mAb and its composition were identified via CEMS, among them 5 glycosylated variants including 4 G0F/G0F and 1 G1F/G1F. This identified mAb also had charge variants, which were successfully separated and identified with the correct MS with CID. The identification of different glycoforms of the NIST mAb was performed using the BioPharma Finder™ 2.0 software package, accurate intact mass for at least 15 glyco-forms was determined with a tolerance of 30 ppm.