Charge heterogeneity characterization of next-generation antibodies using capillary electrophoresis and mass spectrometry

[1]Zuzana Demianova, [1]Fang Wang, [2]Peter Holper, [2]Zoltan Szabo [2]Sahana Mollah
[1]SCIEX, Brea, CA, USA, [2]SCIEX, Redwood City, CA, USA

Introduction

This technical note illustrates a rapid profiling method for charge variant profiling of next-generation antibodies during product development, manufacturing, stability and release testing using capillary zone electrophoresis (CZE) on PA 800 Plus Pharmaceutical Analysis System. Subsequently, the ZipChip System coupled to the TripleTOF® 6600 LC-MS/MS System can be used for deeper characterization of the molecule and identification of the variants.

Next-generation antibodies, such as engineered fusion proteins and bi-specific antibodies carry great potential in clinical applications. Similar to mAbs, they are generally cell-originated and are subject to post-translational modifications that can introduce charge heterogeneity to the molecule. Monitoring of these variants is required throughout manufacturing to assess drug purity and heterogeneity and to ensure the safety and efficacy of the drug.

In earlier work, we demonstrated two simple kits-based assays that provide highly similar charge variants profiles for routine monitoring with CZE-UV and peak identification with CZE-MS for traditional mAb molecules. Here, we used two commercially available molecules (emicizumab-kxwh and blinatumomab) to show that the same sets of tools could also be used for charge variant monitoring and in-depth characterization in next-generation biologics development.

Key features

- Reliable, high-resolution, rapid charge variant profiling (<15 minutes) of next-generation biologics using CZE under native conditions
- Seamless bridge from peak profiling using CZE-UV for routine product monitoring followed by deeper peak characterization with ZipChip MS providing good profile alignment
- Easy and ready-to-use kits-based platforms with minimal sample preparation
- Straightforward data analysis and spectrum reconstruction

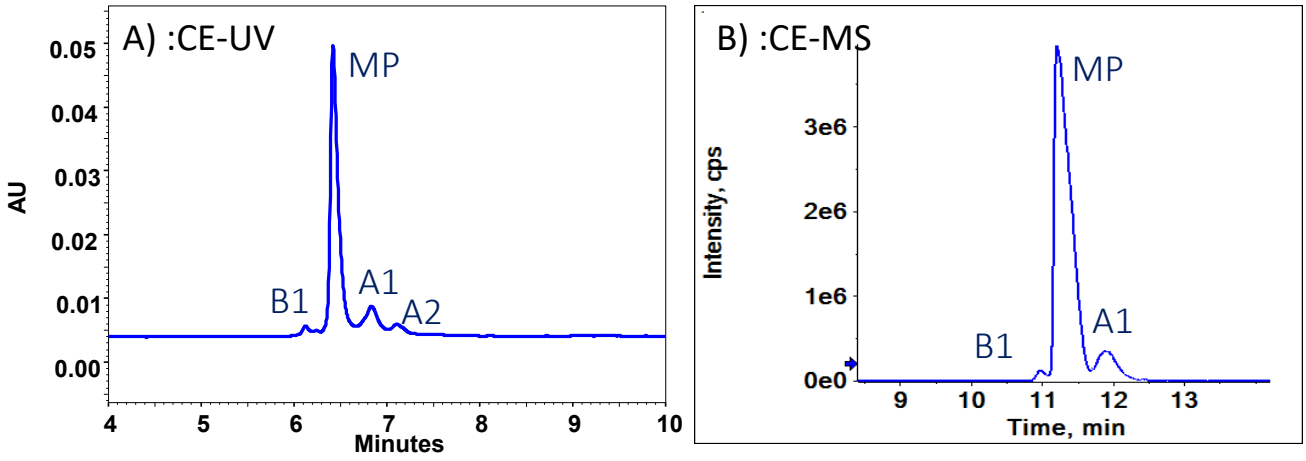

Figure 1. A side-by-side comparison between the charge variants separation with CZE-UV and CZE-MS assay for emicizumab-kxwh. A) CE-UV charge variant profile using CZE Rapid Charge Variant Analysis Kit on a PA 800 Plus Pharmaceutical Analysis System; B) CE-MS charge variant profile using Charge Variant TOF Kit on a ZipChip coupled to the TripleTOF 6600 system. Detailed MS identification is shown in Figure 3.

Methods

Sample reconstitution: Emicizumab-kxwh and blinatumomab antibody were reconstituted to a concentration of 30 µg/µL and 0.2 µg/µL with water, respectively. Stock solutions were stored at -80°C.

Sample preparation: A buffer exchange/sample concentrate step was performed for blinatumomab in both capillary electrophoresis (CE) with optical (UV) and mass spectrometry (MS) detection due to its low concentration. Buffer exchange for emicizumab-kxwh was only performed for CE-MS analysis. emicizumab-kxwh was directly diluted to 1 mg/mL with water from 30 mg/mL stock solution before CE-UV analysis.

Buffer exchange: Before analysis, the antibody stock solutions were desalted and buffer exchanged into sample diluent from the Charge Variant TOF kit (908 Devices) using centrifugal MWCO filters (emicizumab-kxwh 50 kDa and blinatumomab 30 kDa MWCO filter). First, the filters were equilibrated with 500 µL of diluent and spun at 12,000×*g* for 5 min. Each antibody sample was then dispensed into the filter device and spun at 12,000×*g* until the sample was concentrated to a volume of 100 µL or less; five consecutive buffer exchanges were performed by adding diluent up to 500 µL per spin. Emicizumab-kxwh and blinatumomab samples were diluted with diluent to a final concentration of 1 µg/µL and 0.8 µg/µL, respectively.

Table 1. Mass spectrometry methods.

Parameter	Blinatumomab	Emicizumab-kxwh
MS mass range (m/z)	2000 – 5 000	3000 – 6000
MS accumulation time (s)	0.4	
GS 1 (psi)	4	
Curtain gas(psi) :	10	
Polarity:	positive	
Source temperature:	100°C	
Delustering potential (DP)	120 V	
Collision energy (CE)	30 V	50 V
Collisionally activated dissociation (CAD)	7 V	
Sum to bin	100	

Capillary electrophoresis and mass spectrometry: The CE-MS analysis was performed using the ZipChip system (908 Devices Inc.) consisting of an optional autosampler coupled with the TripleTOF 6600 System. A “high-resolution native” (HRN) chip and the Charge Variant TOF kit containing premixed buffers were used here. For each analysis, 2 nL of the sample was injected onto the chip. The separation was performed at 500 V/cm with pressure assistance turned on after 0.5 min using the predefined method “intact proteins.” The total analysis time was set to 15 or 20 min depending on the sample. The Zipchip system is controlled with 908 Devices software, while the TripleTOF 6600 System was controlled with Analyst® TF Software 1.8.1 Detailed MS parameters can be found in Table 1.

Data processing CE-MS: SCIEX OS Software 2.0.0 was used to visualize data, and BioPharmaView™ Software was applied to match the post-translational modification on the emicizumab-kxwh.

Capillary electrophoresis-UV: The capillary electrophoresis instrument used was a PA 800 Plus equipped with a UV detector and a 214 nm bandpass filter (SCIEX P/N 144437). Separations were performed on a pre-assembled bare fused silica cartridge (SCIEX P/N A55625) at 500 V/cm field strength to improve the resolution. The sample was introduced into the capillary via pressure injection for 10 seconds at 0.5 psi. The separation buffer used was from the CZE Rapid Charge Variant Analysis Kit (SCIEX P/N C44790). Instrument control and data acquisition was made using 32 Karat Software 10.0.060

Results and discussion

As biopharmaceutical companies face increasing pressure to decrease the overall drug development timeline, analytical researchers find the need to finalize methods within shorter timelines and with more limited samples. Quickly finding a robust, stability-indicating method that can monitor charge variants is an ongoing analytical challenge. The most popular capillary isoelectric focusing (cIEF) charge variant methods generally analyze biotherapeutics in a denatured state and also face great challenges for peak characterization.[1-2] In general, the identification of peaks in a cIEF profile requires development of orthogonal methods. In contrast, CZE combines the

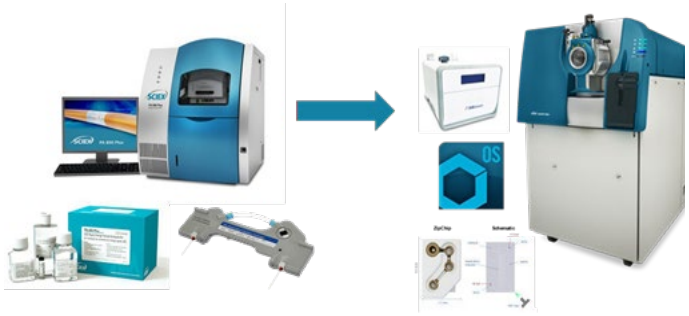

Figure 2. Quick charge heterogeneity profiling of next-generation antibody followed by identification of peaks using the ZipChip system coupled to the TripleTOF 6600 System.

benefits of a native state analysis with the speed and resolution expected of a capillary electrophoresis method. Using a widely universal buffer requires little to no method development, and has a simple sample preparation and a high analytical throughput.[3-4] More importantly, the Charge Variant TOF kit and Zipchip system provide a seamless bridge for intact level peak identification (Figure 2).

Emicizumab-kxwh charge variant characterization: Similar to traditional mAbs, the formulated sample has a concentration of 30 mg/mL, allowing thirty-fold dilutions before being subject to CZE-UV and CZE-MS analysis. The simple buffer exchange step provides better spectral quality (decreased peak/valley ratio) in the CZE-MS results, but no significant change was observed for the CZE-UV analysis.

Blinatumomab charge variant characterization: Due to the low protein concentration in the sample, formulation buffer excipients showed great interference in both CE-UV and CE-MS analysis (Figure 2). After buffer exchange/sample concentration significantly improved charge variant separation observed in CE-UV, the actual protein charge envelop rather than polymer excipients were observed in CE-MS (Figure 3).

Figure 1 shows the side-by-side comparison between CZE-UV and CZE-MS profile for emicizumab-kxwh. Figure 3 panels b and d show the side-by-side comparison between CZE-UV and CZE-MS profile for blinatumomab. Both analyses are carried out according to the kit's instruction without extensive optimization. Similar charge variant profiles were observed for both molecules, demonstrating the possibility of using CZE-UV workflow routinely and performing peak characterization when deemed necessary.

A close look at the MS results indicates that neither of the two next-generation mAbs shows the typical C-terminal lysine variants observed in traditional mAb as the basic variants.[5-6] The dominant acidic variants are consistent with various deamidation numbers (Figure 4 and Figure 5). The glycosylation pattern for emicizumab-kxwh is confirmed at intact MS levels and is labeled in Figure 4. The reconstructed MS for blinatumomab is shown in Figure 5. The lack of mass shift between main peak (MP) and acidic 1 (A1) species indicates the separation between them might occur due to potential conformational difference, which results in differences in surface charges in solution. This is consistent with the native-like conditions in both workflows. Additionally, a charge variant at ~ 3% (A2) also shows good MS spectrum quality, allowing confident spectrum reconstruction and identification.

Both CE-UV and CE-MS workflows can be completed within 30 min for a sample with a total volume of 20 µL samples (concentration range 0.8 – 1.0 µg/µL) for multiple injections, providing a great opportunity for performing charge variant analysis at early stages of product development.

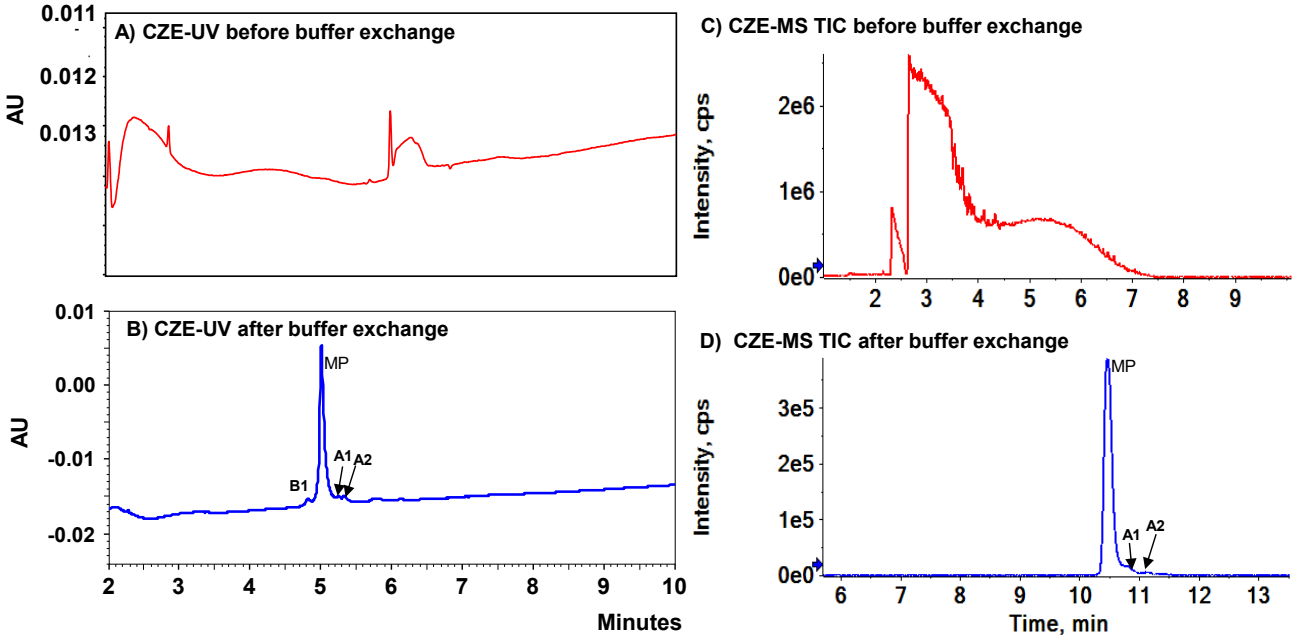

Figure 3. A side-by-side comparison between the charge variants separation with CE-UV and CE-MS assay for blinatumomab. CE-UV charge variant profile A) before and B) after buffer exchange; B) CE-MS charge variant profile using charge variant TOF Kit on a ZipChip coupled to the TripleTOF 6600 system.

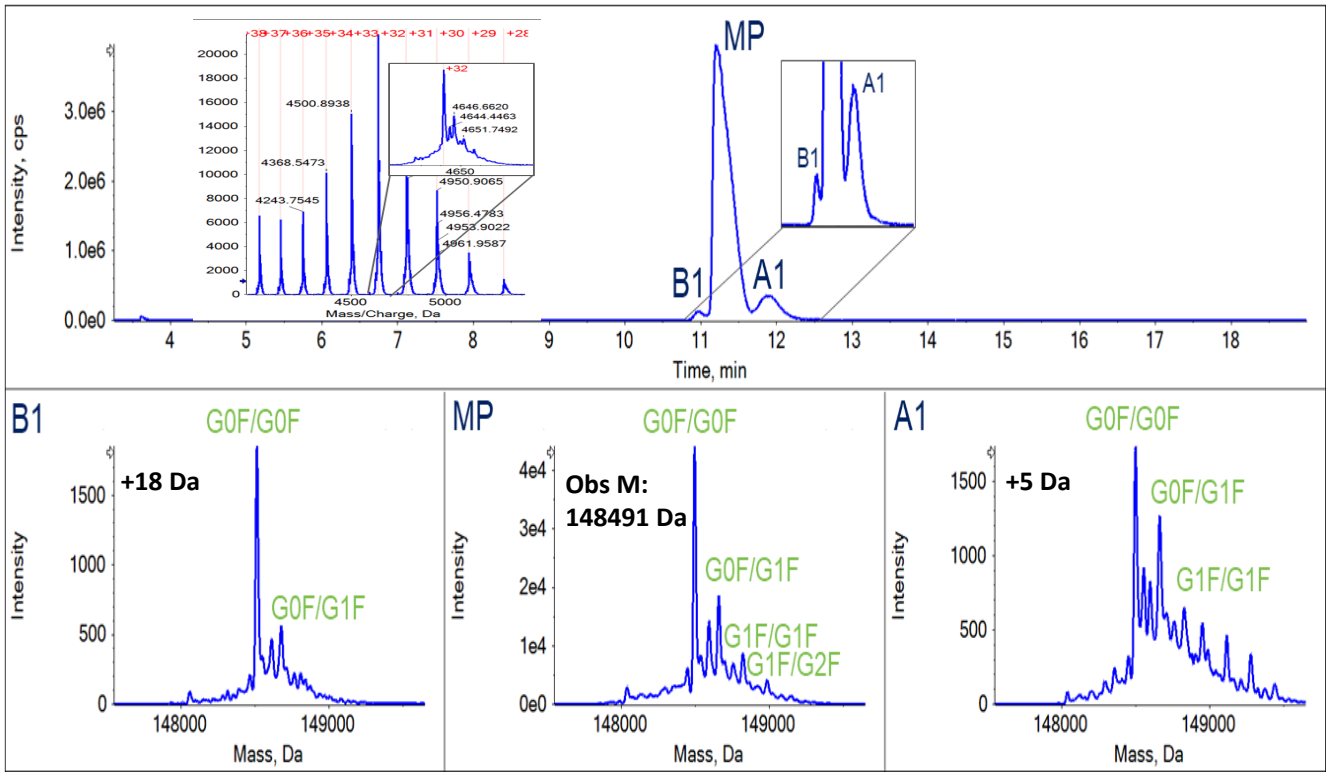

Figure 4. CE-MS charge variant characterization and identification for emicizumab-kxwh. Top), total ion chromatograph of emicizumab-kxwh charge variant with peak labels and a zoomed-in charge envelop as an insert. Bottom): reconstructed mass for each charge variant.

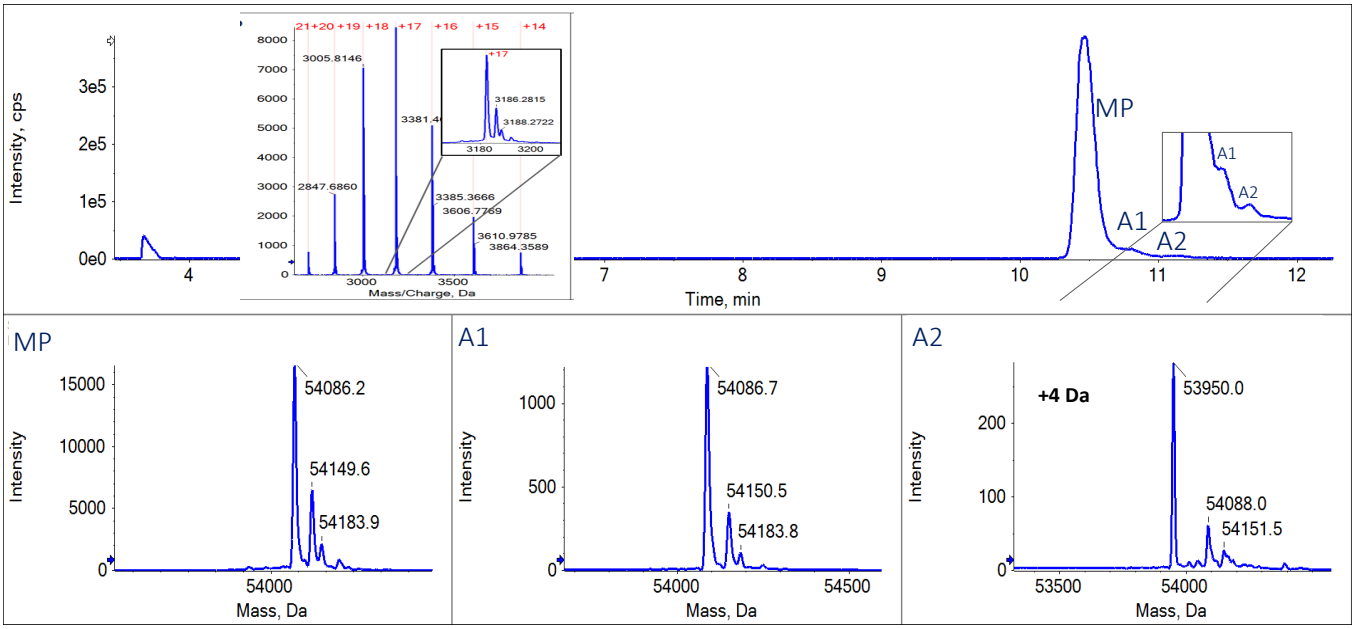

Figure 5. CE-MS charge variant characterization and identification for blinatumomab. Top), total ion chromatograph of blinatumomab charge variant with peak labels and a zoomed-in charge envelop as an insert. Bottom): reconstructed mass for each charge variant.

Conclusions

- Rapid charge variant analysis times (<10 minutes) of next-generation antibody using CE-UV
- Ready to use kits and pre-assembled cartridge enables streamlined analysis
- Seamless bridge from peak profiling with CE-UV to peak characterization with ZipChip CE-MS with good data alignment
- Platform method for intact protein analysis with flexibility for method development and optimization and minimal sample preparation
- Excellent MS sensitivity for identification of low abundance charge variant peaks

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