Charge Heterogeneity Analysis of Intact Infliximab Using CESI-MS and the Neutral OptiMS Cartridge

Fang Wang,[1] Tingting Li,[1] and Esme Candish[2]

1 SCIEX, Brea, CA

2 SCIEX, Framingham, MA

Key features

- Capillary zone electrophoresis (CZE) separation and mass spectrometric (MS) identification of charge variants in a single analysis
- Methodology for separation of basic and acidic infliximab charge variants using an OptiMS cartridge with neutral surface on a CESI 8000 Plus High Performance Separation-ESI Module from SCIEX
- High sensitivity CE-MS (capillary electrophoresis-mass spectrometry) analysis to identify low-level intact mAb (monoclonal antibody) variants
- Identification of charge variants and major glycan species using the SCIEX TripleTOF® 6600 LC-MS/MS System

Figure 1. The CESI 8000 Plus System coupled with the SCIEX TripleTOF 6600 System

Introduction

Monoclonal antibodies (mAbs) are one of the most dominant biotherapeutics. Unlike chemically synthesized drugs, these biotherapeutics are cell originated and are subjected to many different post translational modifications (PTMs) during the process of manufacturing and storage. The most common PTMs include C-terminal lysine truncation, deamidation, glycation and methionine or tryptophan oxidation. These modifications usually lead to changes in the protein's isoelectric point (pI), which presents as multiple charge variants in charge-based purity assays. A comprehensive characterization of the charge variants in the mAb population is crucial, as these variants reportedly affect the safety and efficacy of the biotherapeutics.

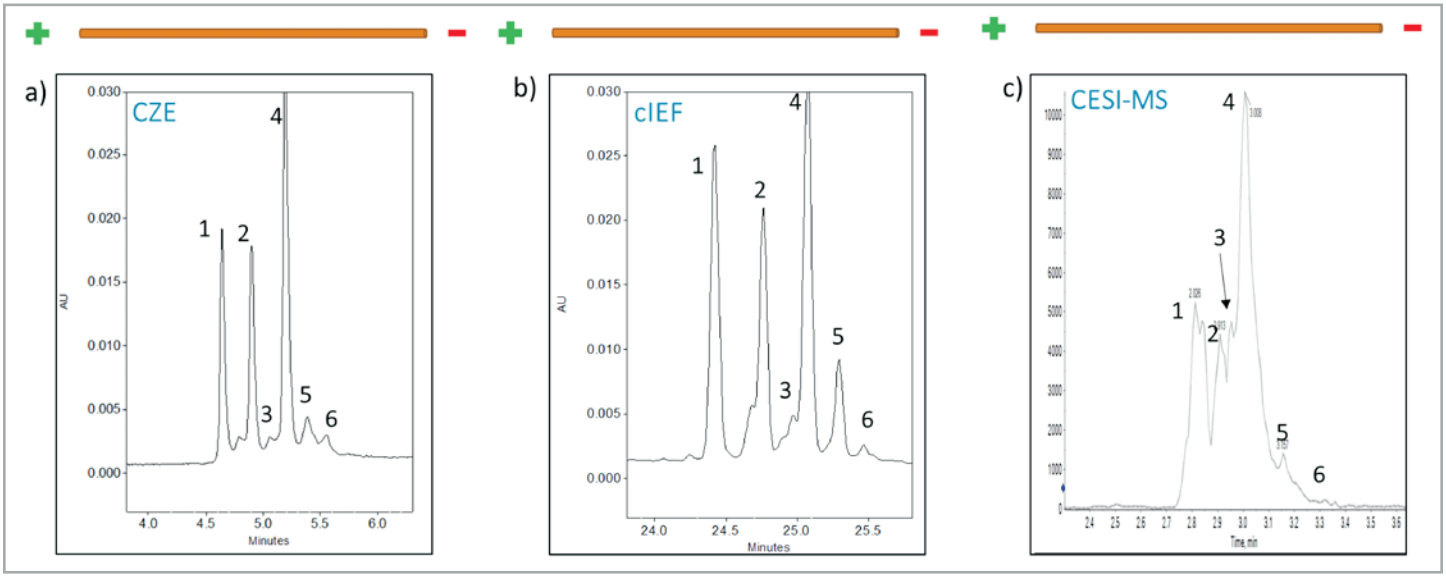

Figure 2. A look at 3 different approaches to charge variants analysis of infliximab: a) UV-based platform CZE method; b) UV-based capillary isoelectric focusing and c) CESI-MS (CZE) with a Neutral OptiMS cartridge.

Many different charge-based separation techniques can be employed to resolve charge variants in the mAb population. Capillary electrophoresis (CE) techniques have been widely applied in the entire life cycle of biotherapeutic development and manufacture. There are 2 platform CE separation mechanisms with optical detection that are routinely used to monitor changes in charge variants that affect product quality. The first, capillary isoelectric focusing (cIEF), separates charge variants on established pH gradient based on their pI. The second, capillary zone electrophoresis (CZE), separates based on the charge to the hydrodynamic size/shape of the molecule. However, while the charge variants can be separated easily with these methods, the characterization of these peaks remains challenging and time consuming. The process typically involves peak fractionation, buffer exchange and fraction confirmation with the original method, followed by RP-LC-MS identification of each fraction. In addition, peak fractionation typically involves an orthogonal technology, which may require additional method development.

The CESI 8000 Plus System allows for the direct coupling of CE and mass spectrometry (MS), providing the possibility of direct identification of peaks separated by CZE. The OptiMS cartridge used on the system achieved the integration of high efficiency and ultra-low-flow CE with electrospray ionization (ESI) into a single dynamic process within the same device. In addition, CESI-MS offers a high-resolution CE and is known for exquisite sensitivity levels due to the nanoflow regime in which it operates. This technical note demonstrates a short CESI-MS method that provides comparable charge variant separation to the widely used CZE and cIEF platform methods, and simultaneously provides information on peak identity and major glycan forms in a single analysis. Infliximab is a commercial antibody drug that is used for treatment of autoimmune diseases. It has a neutral pI value for the main species. This technical note showcases the charge variant analysis of unstressed and stressed infliximab using a CESI-MS system.

Materials and methods

Buffers and reagents

1. Background electrolyte stock (BGE stock): 10% acetic acid prepared by mixing 1 mL of acetic acid with 9 mL of deionized (ddi) water daily
2. Background electrolyte (BGE): 0.3% acetic acid prepared from BGE stock by diluting 1.5 mL of 10% acetic acid stock into 50 mL daily
3. Sample buffer stock: 50 mM ammonium acetate (pH 6.0), also used to perform buffer exchange for protein sample
4. Sample buffer: 5 mM ammonium acetate with 20% methanol prepared by mixing 100 µL of sample buffer stock (prepared in #3) with 200 µL of methanol and 700 µL of ddi water
5. Capillary cleaning solution: 0.1N HCl

Stressed infliximab preparation

Infliximab was diluted into 50 mM ammonium bicarbonate (pH 8.0) and stored in an incubator (maintained at 35–40 °C) for 3 days. Then the sample was buffer exchanged into 50 mM ammonium acetate (pH 6.0) and aliquoted for storage at -20 °C before analysis.

Sample preparation

Infliximab mAb (10 mg/mL) was buffer exchanged into 50 mM ammonium acetate (pH 6.0) with an Amicon 10K filter. The protein concentration should be above 5 mg/mL. The cleanup samples can be stored at 4 °C before use.

Both unstressed and stressed infliximab was then diluted to a final concentration of 0.3–0.5 mg/mL with sample buffer before CESI analysis.

CESI Separation Conditions

Intact infliximab was separated using a CESI 8000 Plus System (SCIEX) equipped with a Neutral OptiMS cartridge (P/N B07368) held at a temperature of 20 °C. The BGE and conductive liquid consisted of 0.3% acetic acid. A sample plug was injected with 1.5 psi for 15 s followed by a BGE injection of 1.0 psi for 10 s. The CESI separation was performed at 30.0 kV with 0 psi for 12 min, followed by 30.0 kV with 0.5 psi (both capillary) for 10 min. The MS acquisition was triggered at 10 min of separation. A 5 min ramp down was included at the end to lower the CE voltage to 1 kV with a 50 psi rinse on both separation and conductive liquid capillary.

Important

- A separation current above 5 µA might cause permanent damage to the separation capillary.

MS conditions

A SCIEX TripleTOF 6600 System with a NanoSpray® III Ion Source and CESI adapter (P/N B07363) was used. The curtain gas was 5 psi and the temperature of the interface was 70 °C. The ESI voltage was set as 1,650 V (calculated as minimum sprayer voltage of the cartridge +150 V). The mass range

employed was 2,000-6,000 m/z, the collision energy (CE) was at 70, the declustering potential (DP) was set at 190, accumulation time was 0.5 s and time bins to sum was set at 80.

Important

- In general, please do not apply >2,000 V to generate electrospray as it may result in capillary damage.

Data analysis

SCIEX PeakView® Software 2.2 and Bio Tool Kit Software were used for data analysis. The MS deconvolution of each peak was performed using an MS range of 140 kDa to 160 kDa.

Results and Discussions

In this technical note, infliximab was employed to demonstrate the capabilities of this CESI-MS method in simultaneously separating and identifying charge variants. The method uses a sample buffer of 5 mM ammonium acetate (pH 6.0) with 20% methanol and mAb concentration at around 0.5 mg/mL. The sample buffer pH is about 1 unit lower than the main peak pI, designed to have the charge variants species in the sample presenting different surface charge, which is used to drive their separation under the applied electrical field. For the separation, 0.3% acetic acid in water was used as the BGE.

Figure 2a, Figure 2b and Figure 2c illustrate three different approaches to achieve charge variant separation of infliximab. Figure 2a employs the CZE method using a BGE containing 400 mM ε− amino caproic acid (EACA), 2 mM triethylenetetramine (TETA) and 0.2% HPMC (pH 5.7), while Figure 2b employs cIEF, a more traditional charge heterogeneity separation scheme using a cIEF kit from SCIEX. Both approaches use UV detection at 214 and 280 nm, respectively, on the PA 800 Plus Pharmaceutical Analysis System from SCIEX.[1, 2,3] In both Figure 2a and Figure 2b, 2 major and minor basic variants (peaks 1, 2 and 3) and 2 major groups of acidic peaks (peaks 5 and 6) in addition to the main peak (peak 4) are separated. The separation profile shown in Figure 2c was achieved using the CESI-MS method described here. Results indicate that similar numbers of peaks along with their relative distributions were observed for all 3 approaches. The deconvoluted spectra of each peak is shown in Figure 3. The caption in blue at the upper left corner of each spectra indicates the MS shift compared to the main peak calculated based on the G0F/G1F species. The caption in green above each spectra indicates the associated glycan pattern.

Peak 4 matches the reported MS of infliximab and presented the highest intensity, and it is therefore identified as the main peak.

Compared to the major form, basic variants in the solution have higher pI, and therefore have more surface charges, while the lower pI acidic variants would have less surface charges in the same buffer environment. The CE separation conditions used in the CESI-MS method allowed for species with higher charges to migrate faster and therefore have a shorter migration time. The deconvoluted MS spectra shown in Figure 3 correspond to each charge variant separated in the electropherogram in Figure 2c. This data reveals that peaks 1 and 2 have a 258 and 129 Da mass shift relative to the main peak and were identified as infliximab with 2 and 1 C-terminal lysine residues, respectively. The migration time of peak 3 indicates they are likely a group of species that is slightly more acidic than the single lysine variant but more basic than the main peak. Additionally, deconvoluted spectra revealed that peak 3 has a 131 Da mass shift relative to peak 2. The higher migration time combined with the mass shift indicate that peak 3 is the deamidated form of the single lysine variant. Peaks 5 and 6 show minor sequential increases in mass, consistent with increased acidity possibly due to an increasing number of deamidation. In addition, 3 major glycoform patterns can be assigned for each charge variant, and the most abundant glycoform is G0F/G1F (Figure 3).

We took this method a step further and applied this workflow to pH and temperature stressed mAbs to evaluate its comparability to the CZE-UV-based method and to further confirm the identity of peak 3 (Figure 2c). Figure 4a and Figure 4b show the profiles of stressed mAbs obtained by both CZE-UV and CESI-MS, respectively. Deconvoluted spectra (not shown) of the 2 marked peaks between 5 and 5.3 min revealed an increased mass of 1–3 Da relative to the main basic variant (~5.4 min), indicating they are likely the deamidated forms of the 2 lysine and 1 lysine variants, respectively. This is consistent with the identification of peak 3 in the unstressed sample (Figure 3). Additionally, the increased height observed for the group of peaks migrating between 5.6 and 6.2 min correspond to fragments with mass around 47 kD (deconvoluted spectra not shown). Similar fragmentation was observed with NIST mAb in our previous work.[4] As expected, this workflow has shown an overall increase in deamidation and degradation products for antibody stressed samples.[5]

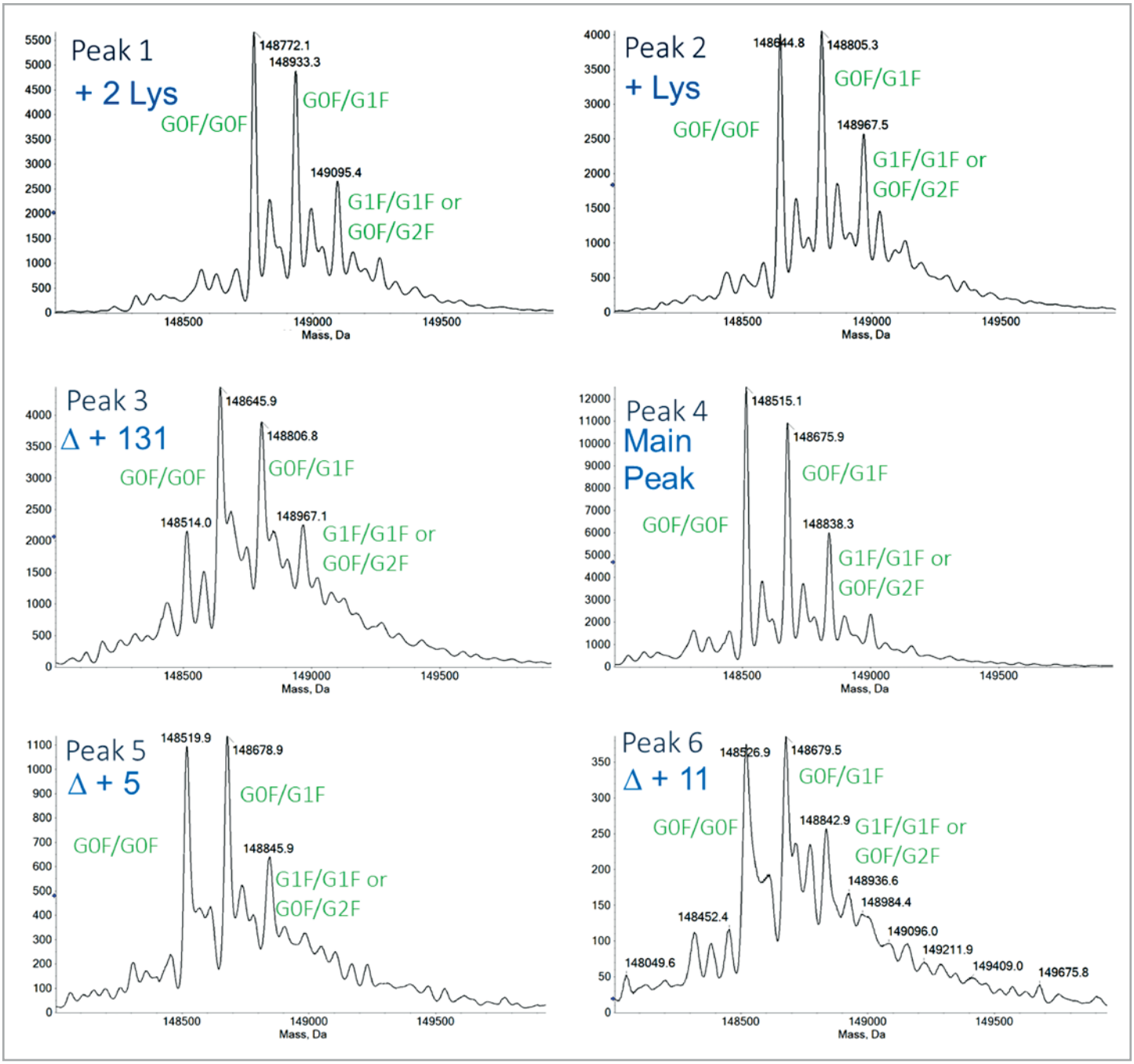

Figure 3. Deconvoluted MS spectra of peaks 1, 2, 3, 4, 5 and 6 labeled in the CESI-MS profile shown in Figure 2c with proposed identification labeled. Peak 3 matches the reported MS of infliximab and presented the highest amount, and it is therefore identified as the main peak. The caption in blue at the upper left corner of each peak indicates the MS shift compared to the main peak and is calculated based on the G0F/G1F species. The caption in green above each deconvoluted MS indicates the associated glycan pattern.

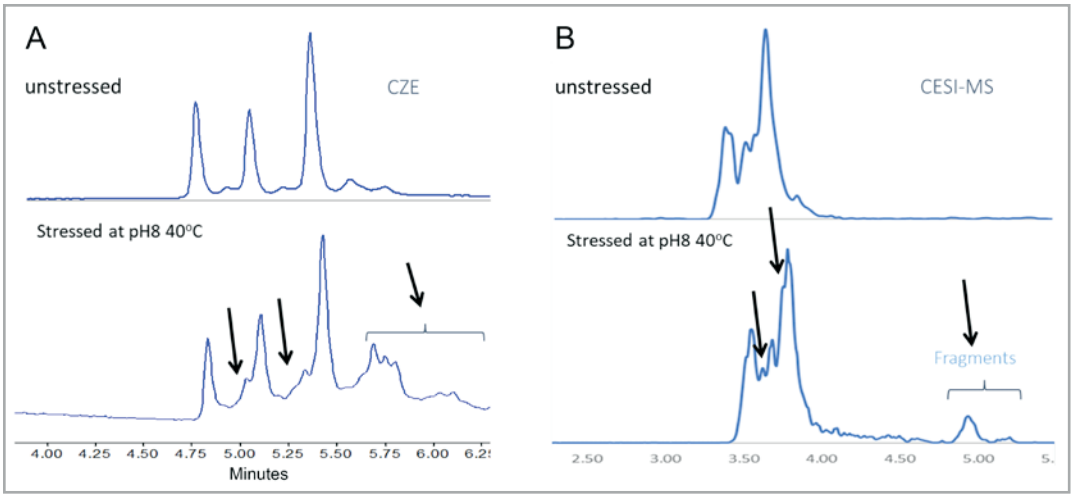

Figure 3. a) Charge variants analysis of unstressed and stressed infliximab on a PA 800 Plus System using the CZE method. b) Charge variants analysis of unstressed and stressed infliximab using CESI-MS (CZE) with a Neutral OptiMS cartridge.

Conclusions

Using infliximab as the test molecule, this technical note illustrates an approach for charge variant analysis of intact mAb using CESI-MS and the commercially available Neutral OptiMS cartridge. A single assay, it provides:

- Comparable separation of the charge variant with the widely accepted platform methods using optical detection, namely CZE-UV and cIEF
- Mass spectra information allowing the identification of different charge variants and possible impurities in the sample
- Characterization of the major glycosylation pattern for each charge variant peak
- Direct detection and identification of new charge variant peaks in samples

References

1. Capillary Isoelectric Focusing (cIEF) Analysis For the PA 800 Plus Pharmaceutical Analysis System Application Guide. SCIEX, RUO-IDV-05-5862-C.
2. Analysis of Monoclonal Antibody Charge Variants by Capillary Zone Electrophoresis. SCIEX.
3. Yan He et al. 2011. Rapid analysis of charge variants of monoclonal antibodies with capillary zone electrophoresis in dynamically coated fused-silica capillary. *Journal of Separation Science*, vol. 34: pages 548–555.
4. Charge Heterogeneity Analysis of Intact NIST mAb Using CESI-MS and Neutral OptiMS Cartridge. SCIEX, RUO-MKT-02-9295-A.
5. H. Liu et al. 2010. Characterization of the stability of a fully human monoclonal IgG after prolonged incubation at elevated temperature. *Journal of Chromatography B*, Volume 837, Issues 1–2, 6 June 2006: pages 35–43.

Headquarters
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