Imaged capillary isoelectric focusing (iCIEF) coupled to high resolution mass spectrometry sharpens depth and width in biopharmaceutical discovery

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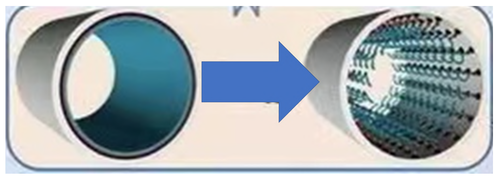
Introduction

The charged heterogeneity of protein drugs (resulting from several mechanisms including chemical degradation, cellular processes, and production conditions during the manufacturing process) requires in-depth structural characterization for critical quality attribute (CQA) assessment to ensure safety, efficacy, and potency. Ion exchange (IEX) chromatography and imaged capillary isoelectric focusing (iCIEF) are important technologies for detecting charged variants of protein drugs. However, the qualitative capability of traditional IEX and iCIEF using UV as the detector is rather limited so high-resolution mass spectrometry (HRMS) is currently the most powerful means of protein identification. Recently, we have been achieving a successful jump in iCIEF-MS to gain both the rapid iCIEF separation and reliable HRMS identification of protein charge variants simultaneously.

New Methylcellulose free column



The MC coated capillary cartridge was applied to iCIEF-MS for characterizing protein charged variants with reliable identification of MS after iCIEF separation, which can greatly simplify the operation steps and prevent the contamination of MS ESI resulting from using routinely coated capillary that usually needs the pre-rinse with MC solution before sample running. This is a breakthrough in iCIEF-MS to greatly improve the compatibility with MS

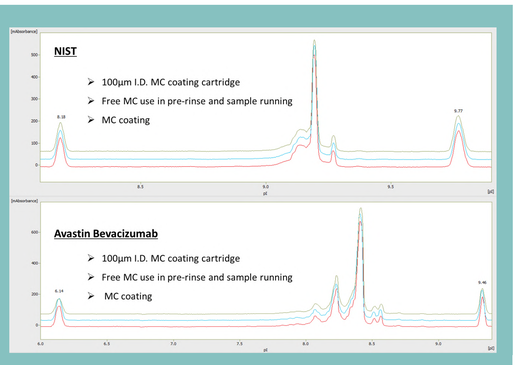


Figure 1. iCIEF-MS using capillary cartridge coated by MC for mAbs.

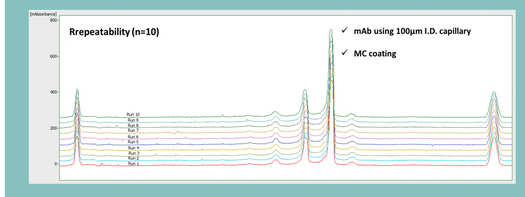


Figure 2. Excellent repeatability of iCIEF separation of mAb on the capillary cartridge coated by MC.

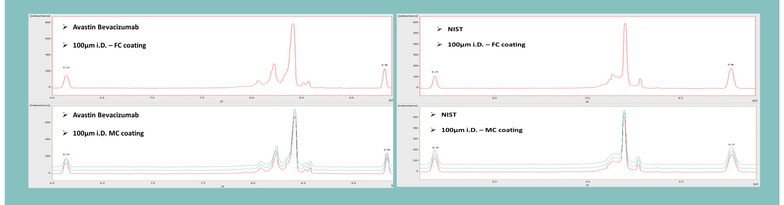


Figure 3. Comparable iCIEF on the capillary cartridges by both FC and MC.

CEinfinite iCIEF-MS solution

CEinfinite iCIEF system can be directly coupled to the mass spec source without modifications. The innovative microfluidic interface improves the sensitivity of identifying protein drug charge variants.

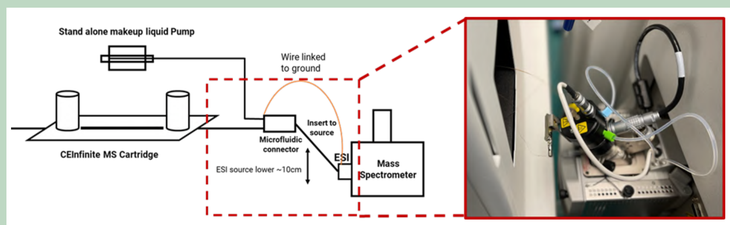


Figure 4. iCIEF-MS schematics

The 'Standard Operating Procedures' (SOP) based on CEInfinite iCIEF, Thermo Fisher quadrupole-orbitrap (QE) mass spectrometer, and Biopharma Finder Software 5.0 is demonstrated for protein charged variant characterization, including step-by-step workflow, complete iCIEF & HRMS experiment conditions, sample preparation and data process.

Figure 5. iCIEF-HRMS workflow for characterizing protein charged heterogeneity. And workflow of Biopharma Finder 5.0 for the processing of data from iCIEF-HRMS.

CEinfinite iCIEF-MS for Bevacizumab and ADC

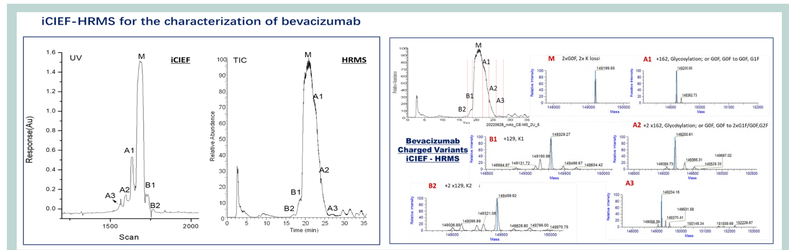


Figure 6a. Electropherogram and total ion chromatogram (TIC) in iCIEF-HRMS analysis of bevacizumab. M: main peak ; A1-3 : acid peak 1, 2 and 3; B1-2: basic peak 1 and 2.

Figure 6b. Structural elucidation of bevacizumab' charged variants by Biopharma Finder 5.0.

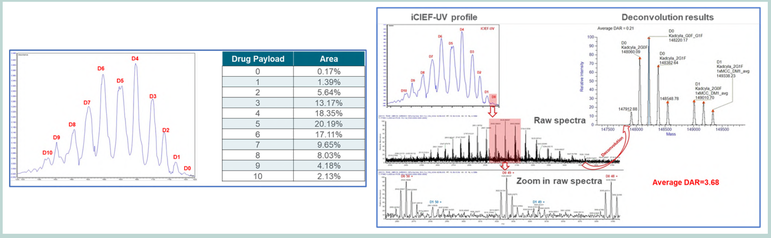


Figure 7a. iCIEF-UV profile of T-DM1 (ADC). About 1.6μg sample was loaded on the column. D0-D10, different drug payloads.

Figure 7b. iCIEF-MS spectra and deconvolution results of T-DM1 (ADC), D0. The complexity of MS spectra was significantly reduced due to iCIEF separation before MS detection.

CEinfinite iCIEF-MS online coupling demonstrated highly reproducible results of for NISTmAb characterization, to warranties the applicability of iCIEF-MS established in QC stage.

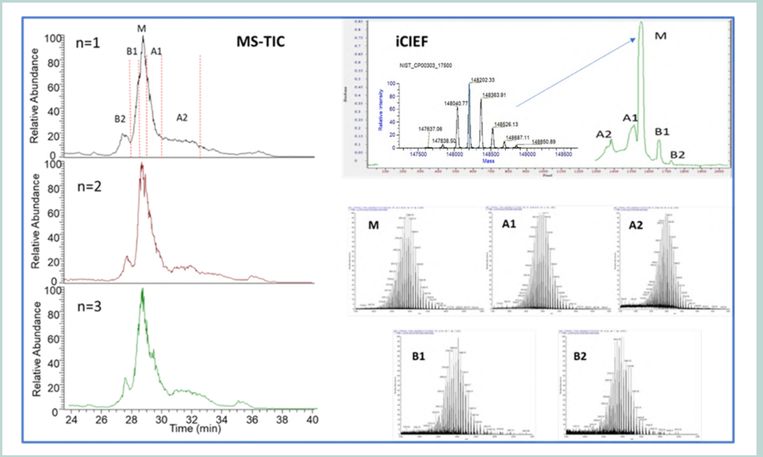


Figure 8. Methodology repeatability of iCIEF-HRMS for Intact NISTmAb (n=3)

Innovations Make Breakthrough Solutions!

Conclusion : AES innovatively developed MS-compatible amphoteric electrolytes and methylcellulose free cartridges using MC-coated capillary instead of its dynamic coating during the analysis, which can realize zero-volatile reagents in the analysis of protein drug charge variants. The whole iCIEF-HRMS analysis based on a seamless MS interface can be solved within 30min, which was employed to characterize a diverse of protein drugs.