IMPROVING ONLINE IEX-MS FOR DOMAIN SPECIFIC CHARGE VARIANT ANALYSIS OF MAB

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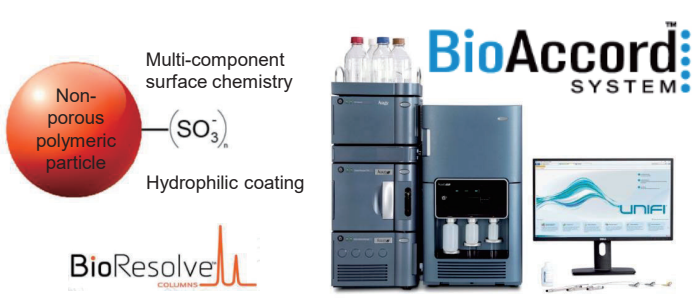
INTRODUCTION

Monoclonal antibodies (mAbs) have been applied as therapeutics for many different diseases. As an intrinsic outcome of their production, these mAbs exhibit heterogeneity corresponding to charge variants that need to be carefully characterized due to their potential impact on drug stability, potency, and efficacy. Ion exchange chromatography (IEX) has been widely adopted for charge variant analysis in the biopharmaceutical industry. However, because of the ubiquitous use of nonvolatile buffers at high ionic strengths, most examples of detailed characterization have relied on time-consuming offline fraction collection or cumbersome multidimensional LC-MS. In this study, a platform online IEX-MS method has been optimized for the charge variant analysis of mAbs that is able to afford robustness and high resolution separations.

METHODS

IEX-MS
Analyses were performed with a UPLC, compact TOF MS instrument, and recently developed BioResolve SCX mAb column, containing a stationary phase based on specialized polymer reactions, hydrophilic surface modification, and DoE formulated sulfonic acid grafting. Online pH detection was performed in separate runs with a GE Healthcare Monitor pH/C 900.



Forced Degradation of Trastuzumab
A sample of Trastuzumab (50 µL @ 20 mg/mL) was buffer exchanged into 100mM sodium phosphate, pH 8.0 using BioRad Micro Bio-Spin® chromatography columns (#732-6221), according to manufacturer protocol. The buffer exchanged Trastuzumab sample was further diluted to 2 mg/mL in 100 mM sodium phosphate, pH 8.0 and was equally split. One half was frozen at –80 ℃ until analysis and the other was incubated at 25 ℃ for 1 week.

IdeS Digestion of Trastuzumab and Other mAb samples
50 µg each Trastuzumab (T0 & 1 week stressed) sample was digested by incubating at 37 ℃ for 30 min with 50 units of FabRICATOR® enzyme (Genovis, A0-FR1-008) in 25 mM NaCl, 25 mM Tris, 1mM EDTA, pH 8.0 (with a final sample concentration of 1 mg/mL). 1 mg/mL samples of nonreduced Trastuzumab (T0 & 1 week), NIST mAb, and Infliximab were also prepared for analysis. 10 µg of each sample was injected for IEX-MS analysis.

SEC-MS Studies on Signal Response
Samples of intact NIST mAb were chromatographed using an ACQUITY UPLC BEH SEC 200 Å 1.7 µm column and various volatile mobile phase conditions. Effects on MS signal were directly observed through detection with a ToF mass spectrometer (Xevo G2 QToF, Waters, Milford, MA).

LC Conditions:
Column: ACQUITY BioResolve SCX mAb 3 µm, 2.1 x 50 mm
Column Temp: 30 ℃
Flow Rate: 0.1 mL/min
Mobile Phases: Ammonium-based dual salt/ pH gradient
Gradient (NR): Hold at 40% B for 1 minute, then 40%-98% B over 20 min (linear), wash at 98% B for 1 min, and re-equilibrate at 40% B for 7 min
Gradient (IdeS): Hold at 2% B for 1 minute, then 2%-98% B over 20 min (linear), wash at 98% B for 1 min, and re-equilibrate at 2% B for 7 min
Total Run Time: 30 min
Injection Volume: 10 µL of 1 mg/mL sample

MS Conditions (RDa Detector):
Capillary Voltage: 1.5 kV
Cone Voltage: 150 V
Desolvation Temp: 350 ℃
Intelligent Data Capture (IDC): Enabled

RESULTS AND DISCUSSION

SEC-MS to Define Method Considerations for IEX-MS
The effects of mobile phase pH and ionic strength were studied by SEC-MS to obtain an orthogonal view on protein ionization efficiency and potential method considerations. Major drops in MS peak areas were observed upon increases from pH 5 to 9. Meanwhile, only minor decreases in signal intensity were observed as salt concentration increased 6 fold. These observations support the rationale behind a dual pH/salt gradient method, and further method optimization resulted in a simplified buffer system with linear pH and conductivity traces for the elution of mAbs and mAb subunits with diverse pIs and retention behavior.

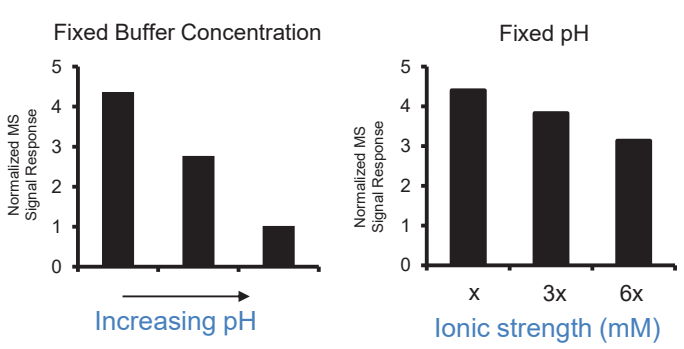


Figure 1. SEC-MS signal response for intact NIST mAb as analyzed under various volatile mobile phase conditions applicable to IEX.

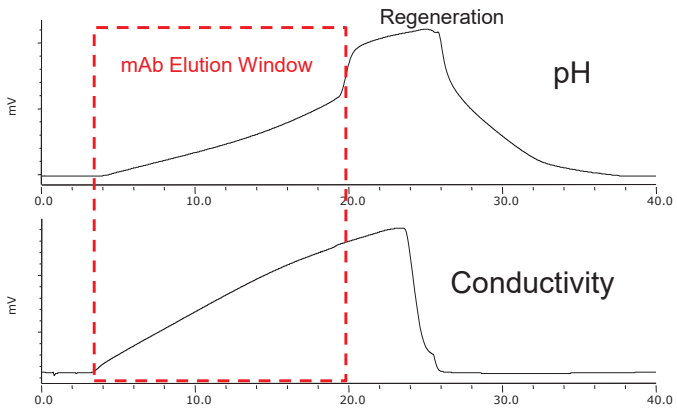


Figure 2. Online pH and conductivity traces obtained for an optimized IEX-MS mobile phase system.

IEX-MS on NIST mAb and Infliximab Charge Variants
To evaluate the applicability of this mobile phase system, we have explored separations of NIST mAb and infliximab charge variants. Example LC-MS data are provided below.

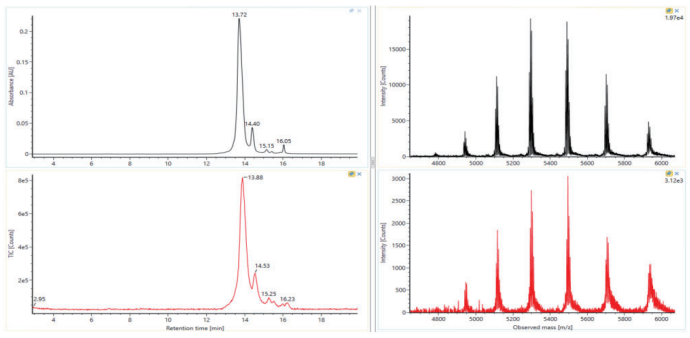


Figure 3. Nonreduced NIST mAb IEX separation and raw MS spectra for main and basic variant peak. A) UV (280 nm) trace; B) TIC trace (m/z 400-7000). The right panel shows combined raw spectra for the C) NIST mAb + 0K and D) NIST mAb + 1K.

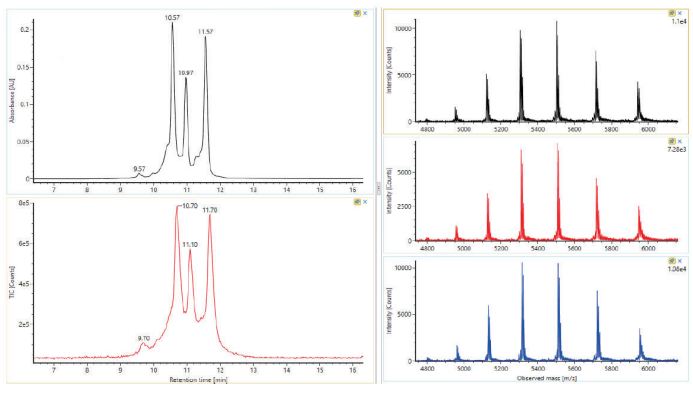


Figure 4. Nonreduced Infliximab IEX separation and raw MS spectra for 3 prominent C-terminal lysine variants. A) UV (280nm) trace; B) TIC trace (m/z 400-7000). The right panel shows combined raw spectra for the C) 0K, D) 1K, & E) 2K species.

IEX-MS Subunit Analysis of Stressed Trastuzumab
Trastuzumab is a monoclonal antibody with well known susceptibility for deamidation at N30T in the light chain and, to a lesser extent N55G in the heavy chain, which have been well characterized. This antibody is easily deamidated under elevated pH conditions, and is therefore an ideal case study for charge variant monitoring via IEX-MS.

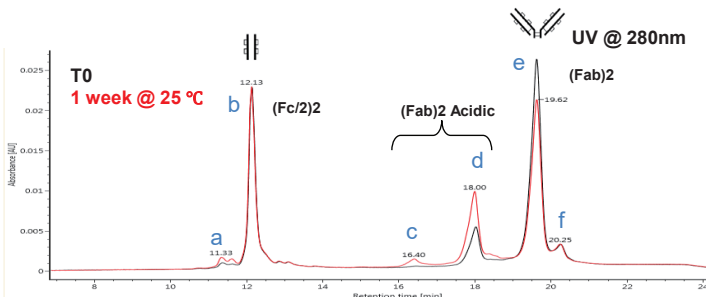


Figure 5. A_{280} chromatogram overlay of T0 and 1 week stressed Trastuzumab, IdeS-digested, and corresponding peak integrations.

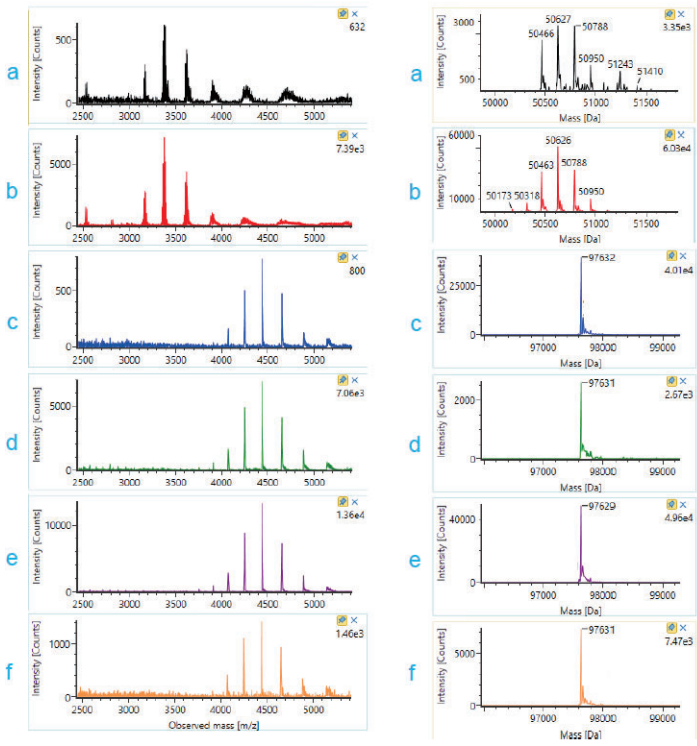


Figure 6. The panel on the left shows combined raw spectra for peaks a-f in Figure 4; the panel on the right displays the corresponding MaxEnt1 deconvolutions of each peak.

Peak	Time (min)	Peak ID	Δ Da[a]	Possible Assignment[b]
a	11.33	$(Fc/2)_2$ Acidic 1	+ 2.0 +292.0	Deamidation + Sialic Acid
b	12.13	$(Fc/2)_2$ Main	-	Unmodified (Fc/2)2
c	16.40	$F(ab')_2$ Acidic 2	+ 5.0	Deamidation
d	18.00	$F(ab')_2$ Acidic 1	+ 1.0	Deamidation
e	19.62	$F(ab')_2$ Main	-	Unmodified (Fab)2
f	20.25	$F(ab')_2$ Basic 1	- 1.0	Possible Disulfide or Conformational Variants

[a] For (Fc/2)2, a representative glycoform is used to calculate □mass
[b] RDa detector mass accuracy spec is < 20 ppm for intact mAb based on NIST mAb standard

Table 1. IdeS digestion IEX-MS results and possible assignments for each peak

CONCLUSION

With a volatile salt mediated pH gradient method, both charge and conformational heterogeneity of IdeS digested mAb subunits, including $(Fc/2)_2$ glycoforms and $F(ab')_2$ deamidation variants, were resolved and characterizable. When paired with a novel small footprint Tof mass detector, this method has thus been seen to be especially promising for monitoring charge variants and facilitating new approaches for subunit level multi-attribute monitoring.

References

1. Yan, Y et al. Analytical Chemistry 2018; 90 (21): 13013-13020
2. Leblanc, Y et al. J Chromatogr B Analyt Technol Biomed Life Sci. 2017;1048:130-139
3. Fussl, F et al. Anal Chem. 2018; 90 (7): 4669-4676
4. Diepold, K et al. PLoS ONE 2012

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