Evaluation of a microfluidic electrophoresis device coupled to an Orbitrap mass spectrometer for the characterization of biotherapeutic proteins.

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ABSTRACT

Purpose: To evaluate the performance of a chip-based electrophoresis device coupled to an Orbitrap mass spectrometer for intact and sub-unit mass analyses and peptide mapping.

Results: Using the ZipChip device, the lysine variants of the NIST mAb were successfully identified at the intact and sub-unit level. For the glycoforms G0F/G1F and G1F/G1F of trastuzumab emtansine, the average DAR values were respectively 3.46 and 3.47, which are in accordance with previously published data. Using the ZipChip device, a sequence coverage in excess of 97% was observed for the light and heavy chains after a 10 min peptide mapping experiment.

INTRODUCTION

The discovery and development of biotherapeutics continues to accelerate. The complexity of these agents and the increasing requirements to characterize them for both safety and efficacy places a large burden on the analytical scientists tasked with these challenging demands. Intact and sub-unit mass analysis as well as peptide mapping are widely used to get insights on biotherapeutics. Often these assays are LC-MS based, but the development of a new microfluidic capillary electrophoresis device could offer a fast and sensitive orthogonal mode of separation. Here we evaluate the performance of a chip-based electrophoresis device coupled to an Orbitrap mass spectrometer for some of the major workflows used to characterize biotherapeutics

MATERIALS AND METHODS

Sample Preparation

The analyzed samples were the NIST mAb and trastuzumab emtansine. For intact mass analysis, the sample was simply diluted in water prior to analysis.

For sub-unit mass analysis, the sample was first diluted in Tris HCl 0.1M and digested with the IdeS protease. Subsequently, the sample was denatured with Guanidine 8M and reduced with DTT. The final step is a buffer exchange with the dilution buffer provided by 908 Devices in the peptide kit.

For peptide mapping, NIST mAb was denatured in guanidine HCl and Tris followed by reduction and alkylation with DTT/IAA. The alkylation was quenched with DDT following buffer exchange in 50 mM Tris using BioSpin™ 6 columns (Bio-Rad Laboratories). Trypsinization was performed at 37 ºC for 30 min and quenched by lowering the pH with formic acid. Finally, the sample was diluted with the peptide dilution buffer provided in the 908 devices peptide kit.

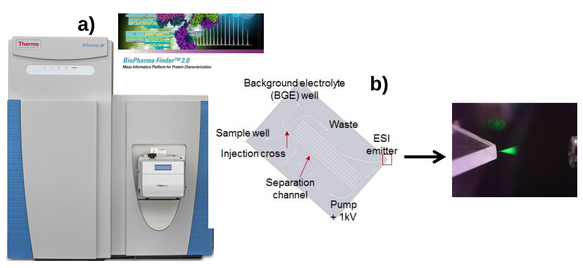
Methods

Microfluidic electrophoresis
The ZipChip™ HR chip from 908 Devices (Boston, MA) was used for all experiments. For intact and subunit mass analysis and peptide mapping, field strengths of 500V, 220V and 400V were respectively used.

Mass Spectrometry
Mass spectrometer: A Thermo Scientific™ Q Exactive™ HF MS operated in high mass range (HMR) mode was used for the intact mass analysis and in standard mode operation for peptide mapping. A Thermo Scientific™ Q Exactive™ Plus in protein mode was used for the sub-unit mass analysis.

Data Analysis
For all of the experiments, Thermo Scientific™ BioPharma Finder™ 2.0 software was used.

Figure 1. a) Data were collected using the ZipChip device coupled to a Q Exactive Plus or Q Exactive HF instrument and processed with BioPharma Finder software. b)The ZipChip HR is a twenty two centimeter etched channel with a neutral hydrophilic coating with an integrated nanoelectrospray emitter



RESULTS

A) Intact Mass Analysis

1) NIST mAb

Figure 2. a) Electropherogram of NIST mAb (0.1 ug/uL). b) Spectra of the NIST mAb and the lysine variants. c) Zoom in the region of the charge +30 ions.

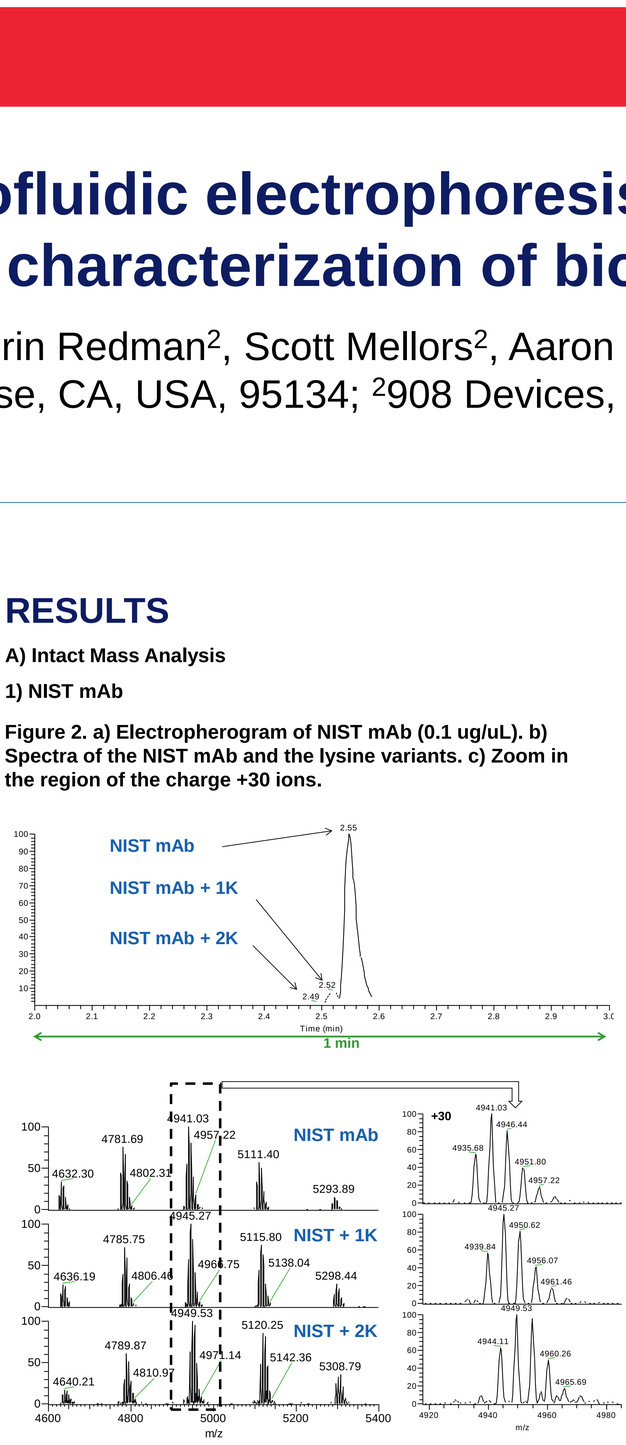


Table 1. Deconvoluted masses of the NIST mAb and lysine variants major glycoforms.

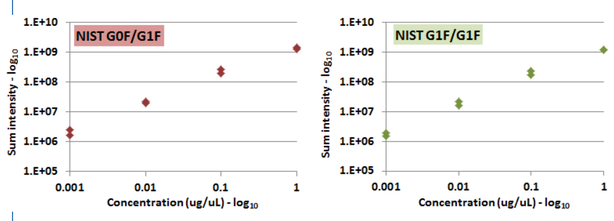
Protein Name	Modification	Average Mass	Theoretical Mass (Da)	Matched Mass Error (ppm)	Sum Intensity	Relative Abundance	Fractional Abundance
NIST	G0F/G0F	148038.8	148037.1	11.5	1.28E+09	58.82	16.61
NIST_plus1K		148165.6	148165.3	1.9	7.66E+07	3.51	0.99
NIST_plus2K		148295.4	148293.5	13.1	1.04E+07	0.48	0.14
NIST	G0F/G1F	148200.0	148199.3	5.0	2.18E+09	100.00	28.24
NIST_plus1K		148327.4	148327.4	0.1	1.39E+08	6.36	1.80
NIST_plus2K		148456.9	148455.6	9.0	1.56E+07	0.72	0.20
NIST	G1F/G1F	148362.1	148361.2	6.0	1.82E+09	83.26	23.51
NIST_plus1K		148489.7	148489.4	2.5	1.18E+08	5.40	1.53
NIST_plus2K		148618.4	148617.5	5.6	1.51E+07	0.69	0.20
NIST	G1F/G2F	148522.7	148523.5	5.8	1.00E+09	46.00	12.99
NIST_plus1K		148652.3	148651.7	3.6	5.97E+07	2.74	0.77
NIST_plus2K		148779.4	148779.9	3.1	8.07E+06	0.37	0.10
NIST	G2F/G2F	148684.6	148685.7	7.2	4.84E+08	22.21	6.27
NIST_plus1K		148811.9	148813.9	13.4	2.90E+07	1.33	0.38
NIST_plus2K		148942.0	148942.0	0.4	3.49E+06	0.16	0.05

0.65 nL of the NIST mAb at 0.1 ug/uL was injected and the three lysine variants are almost baseline separated. The shift in mass can be observed in the zoom in the region of the +30 charge state ions. The fast and almost base line separation of the lysine variants using the ZipChip combined with the high mass accuracy of the Orbitrap analyzer translated to the identification of 15 different glycoforms with a mass error below 14 ppm.

Table 2. Deconvoluted masses and intensities of the NIST mAb for the glycoforms G0F/G1F and G1f/G1F at different concentrations.

Injection Volume = 0.65 nL									
NIST G0F/G1F					NIST G1F/G1F				
Concentration (ug/uL)	Average Mass (Da)	Theoretical Mass (Da)	Matched Mass Error (ppm)	Sum Intensity	Concentration (ug/uL)	Average Mass (Da)	Theoretical Mass (Da)	Matched Mass Error (ppm)	Sum Intensity
0.001	148199.55	148199.26	2.0	1.66E+06	0.001	148362.39	148361.20	8.0	1.55E+06
	148198.63		4.2	2.48E+06		148361.96		5.1	1.91E+06
0.01	148199.59	148199.26	2.2	2.26E+07	0.01	148362.02	148361.20	5.5	2.23E+07
	148199.47		1.5	1.95E+07		148361.14		0.4	1.63E+07
0.1	148200.60	148199.26	9.1	2.70E+08	0.1	148361.93	148361.20	4.9	2.35E+08
	148200.36		7.4	1.96E+08		148362.41		8.2	1.71E+08
1	148199.01	148199.26	1.7	1.43E+09	1	148362.74	148361.20	10.4	1.18E+09
	148199.55		4.1	1.29E+09		148360.64		3.8	1.20E+09

Figure 3. graphs representing the sum of the intensity of the deconvoluted masses versus the concentration for the NIST mAb glycorforms G0F/G1F and G1F/G1F.



The low sample consumption and high sensitivity of the intact mass analysis assay with the ZipChip are illustrated with the dilution series of the NIST mAb. Even at 0.001 ug/uL when only 0.65 nL are injected, glycorforms G0F/G1F and G1F/G1F are identified at less than 10 ppm.

2) Trastuzumab Emtansine

Figure 3. Data visualization in BioPharma Finder 2.0 software of the lysine-linked ADC.

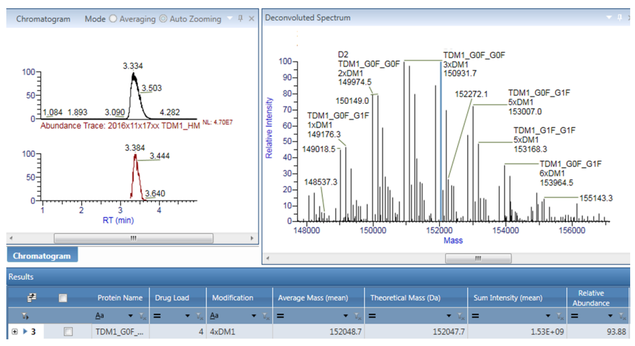
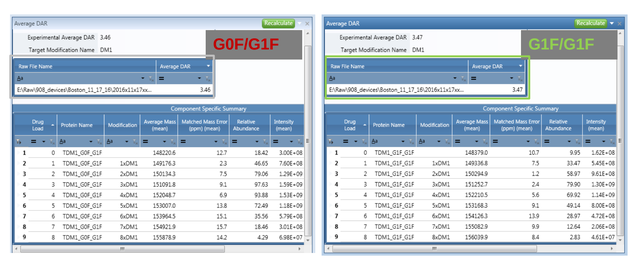


Table 3. Average DAR calculation for glycoforms G0F/G1F and G1F/G1F of trastuzumab emtansine



Around 2.5 ng of Trastuzumab emtansine (1ug/uL) were injected on the microfluidic channel and all of the different forms of trastuzumab emtansine were separated in less than 40 seconds. Trastuzumab emtansine was only diluted in water and the average DAR values for different glycoforms were successfully determined. For the glycoforms G0F/G1F and G1F/G1F, the average DAR values are respectively 3.45 and 3.47 which is consistent with previous published data [1]. Even for this highly complex sample, the mass error for the different DAR is below 16 ppm.

B) Sub-Unit Mass Analysis

Figure 4. a) Electropherogram of NIST mAb and lysine variant sub-units. b) - c) Electropherogram of the components representing G0F scFc for the NIST mAb and the lysine variant.

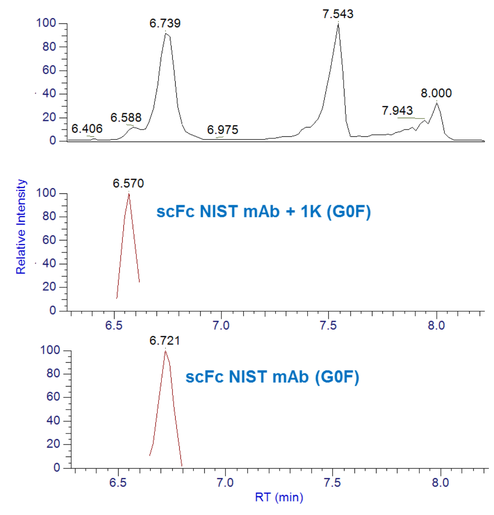


Figure 5. Deconvoluted spectrum of the of the NIST mAb and lysine variants sub-units.

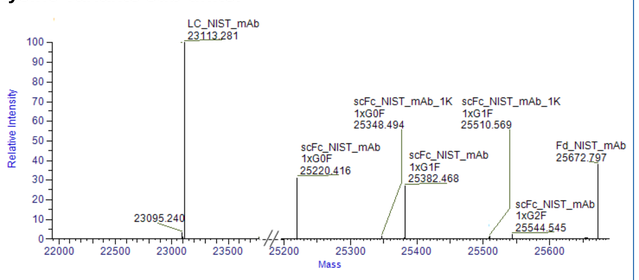


Table 4. Deconvoluted masses of the of the NIST mAb and lysine variants sub-units.

Protein Name	Modification	Monoisotopic Mass	Theoretical Mass (Da)	Matched Mass Error (ppm)	Sum Intensity	Relative Abundance	Fractional Abundance	RT (min)
LC NIST mAb		23113.281	23113.304	1.0	3.62E+07	100.00	45.61	7.54
Fd NISTmAb		25672.797	25672.807	0.4	1.38E+07	38.09	17.37	8.00
scFc NIST mAb	1xG0F	25220.416	25220.463	1.9	1.15E+07	31.60	14.41	6.72
	1xG1F	25382.468	25382.516	1.9	9.93E+06	27.41	12.50	6.72
	1xG2F	25544.545	25544.569	1.0	8.45E+05	2.33	1.06	6.72
scFc NIST mAb_1K	1xG0F	25348.494	25348.558	2.5	5.15E+05	1.42	0.65	6.57
	1xG1F	25510.569	25510.611	1.6	5.09E+05	1.40	0.64	6.57

The scFc of the NIST mAb and the lysine variant have different migration times and were identified at less than 2.5 ppm.

C) Peptide mapping

Figure 6. Electropherogram of NIST mAb tryptic digest after processing in BioPharma Finder 2.0 software.

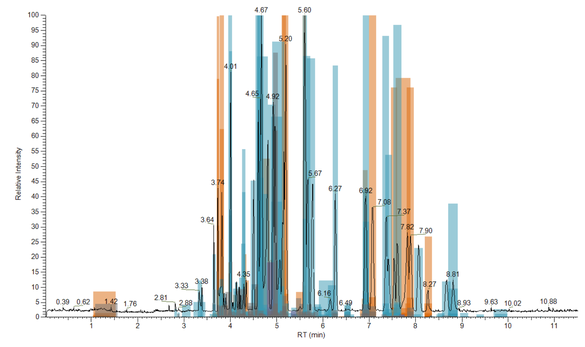
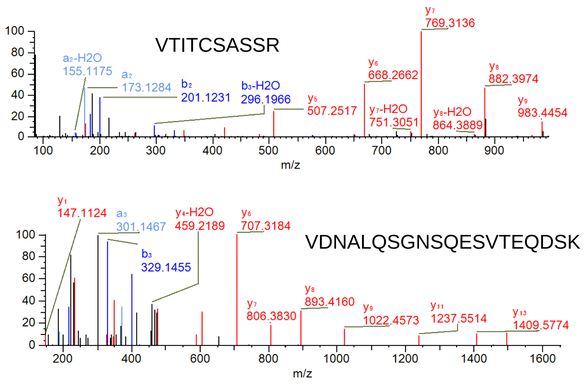


Table 5.Sequence coverage after processing in BPF 2.0.

Proteins	Number of MS Peaks	MS Peak Area	Sequence Coverage	Abundance
NSIT mAb light chain	141	26.4%	100.0%	41.67%
NIST mAb heavy chain	339	60.5%	97.6%	56.35%
Unidentified	1441	12.6%		

Figure 7. Examples of MS/MS acquired on the Q Exactive Plus instrument after separation on the ZipChip device.



High sequence coverage was obtained for the light and heavy chains with MS/MS confirmation after less than 10 min separation on the ZipChip.

CONCLUSIONS

- ZipChip-based electrophoresis combined with the Q Exactive mass spectrometer provides a fast and sensitive intact mass analysis assay.
- ZipChip-based electrophoresis can separate the lysine variants of the NIST mAb at the intact and sub-unit level.
- Lysine-linked ADCs are very heterogeneous samples and can be successfully analyzed without sample pre-treatment by ZipChip-based electrophoresis.
- Above 97% sequence coverage for the light and heavy chains of the NIST mAb are observed after a fast electrophoretic separation and MS/MS confirmation.

REFERENCES

1. Marcoux et al., Protein Sci. 2015 Aug;24(8):1210-23

TRADEMARKS/LICENSING

PO72418-EN 0617S