ULTRA-LOW ESI-MS FLOW RATES MAKE A DIFFERENCE IN THE ANALYSIS OF BIOTHERAPEUTICS

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ABSTRACT

Biopharmaceuticals, especially therapeutic monoclonal antibodies, have emerged as a very promising new generation of protein drugs, but concomitantly represent new bioanalytical challenges for the field. While these large biomolecules require comprehensive structural characterization, their heterogeneity and complexity in most instances are beyond the application domain of the analytical techniques available today. Integration of capillary electrophoresis with electrospray ionization in a single dynamic process (termed CESI) coupled with high resolution mass spectrometry holds the promise to fulfill this gap, even at the intact protein level. Some of the main advantages of CESI-MS are the ability to produce stable electrospray at ultra-low flow rates (5-20 nL/min range) in a robust and reliable manner. In this presentation, the effect of CESI flow rate on ionization efficiency, ion suppression and detection sensitivity will be discussed. Our intact therapeutic antibody analysis results demonstrated that the sensitivity of CESI-MS was increased by an order of magnitude with the decrease of the flow rate from 250 nL/min to 20 nL/min. On the other hand, ultra-low flow rates significantly (2.5x) reduced the ion suppression effect in respect to samples containing both highly and weakly ionizable analytes of biotherapeutic interest.

SAMPLE PREPARATION

Mixtures of maltotetraose (M = 684.12 g/mol) and neurotensin (M = 1674.04 g/mol) were prepared in equimolar concentration of both analytes at 10^{-5} mol/L in a mixture of 10 mM aqueous ammonium acetate and methanol (1:1 by volume). For the intact protein analysis Humira was analyzed at a concentration of 3 µM in 5% formic acid solution.

INSTRUMENTATION

CESI 8000 conditions

Uncoated capillary
Pressure infusion
Capillary i.d.: 30 µm
Capillary length: 91 cm

CESI 8000 was hyphenation with Thermo LTQ and Q-Exactive mass spectrometers, respectively.

ION SUPPRESSION

The liquid flow rate in electrospray ionization (ESI) determines the initial droplet size, thus plays an essential role in the efficiency of the spray process. It has also been reported that genuine nano-ESI, where ion suppression is sufficiently low or even negligible, is only available under a given flow rate limit of 20 nL/min [1]. While previous studies focused on the determination of such a flow rate limit1 with regular ESI spray settings, this is the first study to investigate this effect with the porous sprayer (CESI setup). CESI is the integration of capillary electrophoresis (CE) and electrospray ionization (ESI) into a single dynamic process[2]. In this presentation, flow rates were controlled by the built-in pump of a CESI 8000 instrument and were accurately determined using the Hagen-Poiseuille equation:

$$\Delta P = \frac{8\mu L\dot{V}}{\pi r^4}$$

where ΔP is the pressure difference, μ is the dynamic viscosity, L is the total length of the capillary, V is the volumetric flow rate and r is the radius of the capillary.

First the ion suppression phenomenon was systematically studied using a well-defined oligosaccharide – peptide mixture. Maltotetraose represented an uncharged oligosaccharide, which is considered a weakly ionizable analyte. Neurotensin, on the other hand, is an easily protonated, 13 amino acid peptide. Figure 1 shows the signal intensity ratios as the function of the increasing flow rate. As one can observe, the lower the flow rate the lower the neurotensin / maltotetraose intensity ratio, i.e., lower the ion supression. As a first approximation we consider that lower flow rates produce smaller initial droplet size thus the formed free ions have a more diluted chemical environment where analyte – analyte interactions are less pronounced. Consequently, the inherent charge of neurotensin has no such a significant effect on the individual ionization efficiency of the maltotetraose molecules.

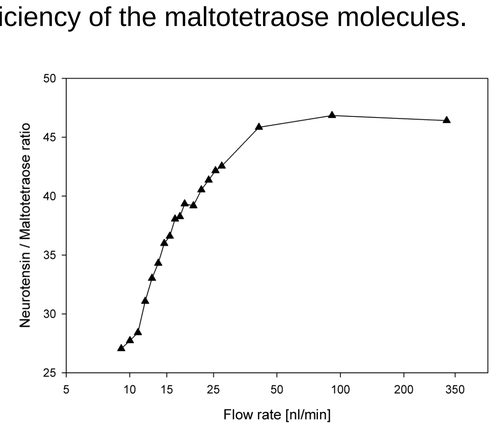

Figure 1 Signal intensity ratios at different flow rates between neurotensin (NT) and maltotetraose (MT) calculated as (NT^{+1} + NT^{+2} +NT^{+3}) / MT^{+1}.

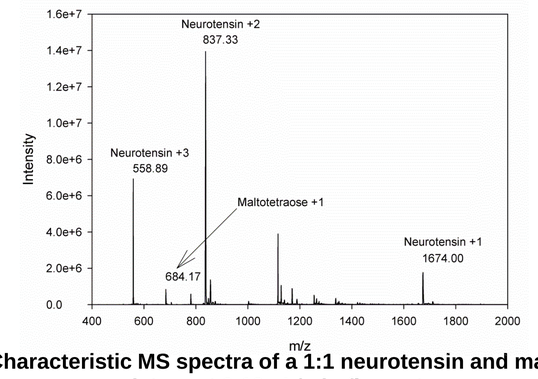

Figure 2 Characteristic MS spectra of a 1:1 neurotensin and maltotetraose mixture at 9.12 nL/min flow rate.

Table 1. Measured signal intensity ratios of neurotensin and maltotetraose at different flow rates.

Infusion pressure [psi]	Averaged spectra	Intensity				Flow rate [nL/min]	Ratio
		Maltotetraose^{+1}	Neurotensin^{+1}	Neurotensin^{+2}	Neurotensin^{+3}		
1	244	838711	1771219	13993410	6921331	9.12	27.0
1.1	221	1346418	2499806	23176639	11654660	10.03	27.7
1.2	251	1652481	2973308	27676186	16300594	10.94	28.4
1.3	251	1726713	3077521	30871399	19691281	11.85	31.1
1.4	250	1770887	3087956	31746342	23643594	12.76	33.0
1.5	250	1781625	3090007	31724453	26307249	13.67	34.3
1.6	251	1863803	3132952	34562099	29372344	14.59	36.0
1.7	251	1892630	3188725	34905682	31172937	15.5	36.6
1.8	250	1923802	3194381	37396024	32614608	16.41	38.1
1.9	251	1946721	3221775	37989072	33272951	17.32	38.3
2	244	1954290	3290323	38538087	35045213	18.23	39.3
2.2	209	1978186	3290004	38083873	36135105	20.06	39.2
2.4	200	1965926	3300695	39336762	37043062	21.88	40.5
2.6	254	1950453	3360795	39678990	37629612	23.7	41.4
2.8	229	1937822	3391312	39894456	38409220	25.53	42.2
3	252	1930555	3368449	40302172	38470789	27.35	42.5
4.5	226	1874585	3453981	41314039	41162057	41.02	45.8
10	251	1996810	3802068	42619829	47102546	91.16	46.8
35	250	1062271	3472131	25254150	20571653	319	46.4

Intact mAb analysis

Robust, comprehensive and reproducible characterization of monoclonal antibody therapeutics is crucial in the biopharmaceutical industry. This can represent a challenge when sample availability is limited[3, 4]. MAbs are subject to co- and post-translational modifications, which results in e.g., glycosylation micro-heterogeneity that may affect antigenicity and immunogenicity5. MAb analysis is usually performed at the peptide level after tryptic digestion (level 3 analysis) and requires complex sample preparation steps, which could hinder some of the structural details6. CESI offers mAb analysis at the intact protein level (level 1 analysis) using only very small amounts of samples. Figure 3 compares the MS spectra of the analysis of an intact protein therapeutics Humira, by simple infusion using the CESI sprayer. The spectra in Figure 3A was obtained by infusing 3 µM Humira sample with a flow rate of 20 nL/min. Figure 3B shows the same but at 12.5 fold higher flow rate (250nL/min). Deconvolution of the spectra was accomplished by an in-house developed script.

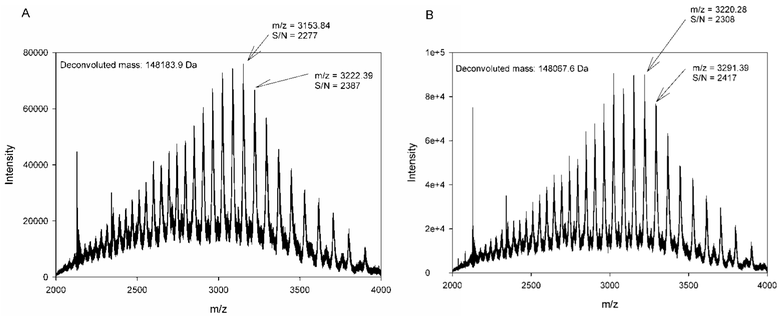

Figure 3 Humira infusion at 20 nL/min (A) and 250 nL/min (B) flow rate. 20 min integration was used to generate MS spectra.

SENSITIVITY

The sensitivity of the CESI-MS setup for intact protein analysis was evaluated by comparing the MS spectra measured at different flow rates. The qualitative analysis of the spectra in Figure 3 revealed no significant differences between the observed spectra at 20 and 250 nL/min flow rates. Assuming same infusion times, lower flow rate requires less sample amount, which is important when the sample availability is limited. Furthermore, the decreased flow rate dramatically increased the sensitivity of the analysis. Figure 4 shows the observed counts relative to the unity (one mole) amount of the infused analyte. As the figure depicts, at 20 nL/min flow rate the detection sensitivity increased by an order of magnitude compared to the higher flow rate of 250 nL/min.

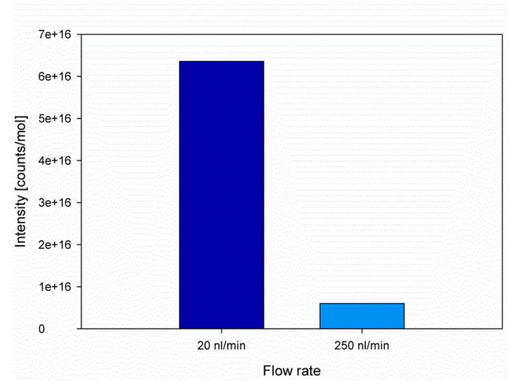

Figure 4 Normalized signal intensities at 20 nL/min (left) and 250 nL/min (right) flow rates. Sample: 3 µM Humira.

Conclusions

Increased sensitivity. CESI-MS at a flow rate of 20 nL/min showed increased sensitivity by an order of magnitude compared to higher flow rate (250 nL/min).

Reduced sample requirement. CESI-MS at a flow rate of 20 nL/min produces the same spectrum quality at the intact protein level as at a flow rate of 250 nL/min.

Decreased ion suppression. Ultra-low flow rates (< 20 nL/min) significantly (2.5x) reduced the ion suppression effect in respect to samples containing both highly and weakly ionizable analytes.

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TRADEMARKS/LICENSING