Optimization and performance characterization of a microscale FAIMS chip coupled to an Orbitrap mass spectrometer

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1. Introduction

Field-asymmetric waveform ion mobility spectrometry (FAIMS) has been shown to provide additional discrimination of ions prior to mass analysis. Macro-scale FAIMS devices have been used to “fractionate” samples after ionisation. Analysis of mass spectra resulting from multiple fractions has been shown to potentially increase sequence coverage [1]. Compared with macro-scale FAIMS systems, chip-based microscale FAIMS devices can provide significantly faster separation and are more compatible with nanospray ionisation sources. Here we describe the integration of a microscale FAIMS chip (Owlstone Ltd, Cambridge, UK) to Thermo Scientific™ LTQ Orbitrap™ mass spectrometer or Thermo Scientific™ Exactive™ Orbitrap mass spectrometer.

2. RF Waveform

An important consideration was to ensure that the electrical impedance characteristics of the interface were within the constraints required for an effective FAIMS waveform. Microscale FAIMS chip mass spectrometer interfaces previously reported in the literature operated at 27.12MHz [2]. The Thermo-compatable interface caused slight additional load on the RF driver circuit, which resulted in reduced voltage output. Reducing frequency to 26.0MHz corrected this, allowing for a more similar waveform magnitude and duty cycle (Figure 1).

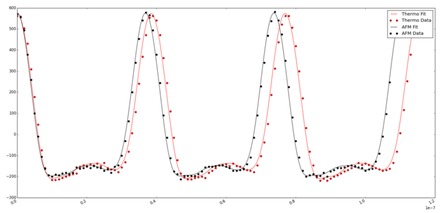

Figure 1. Comparison of target and actual interface waveforms.

3. Mechanical Interface

Key goals of the design were to ensure good desolvation of ions before entering the chip, to minimize transmission losses and to deliver a carrier gas velocity profile optimized for high separation performance.

In the first design iteration, the chip was positioned close to the interface inlet, with desolvation capability provided by a curtain gas (Figure 2). With nanospray, ion abundance levels were around 14% of that observed with no FAIMS for singly protonated MRFA peptide (*m/z* = 524) but < 1% for singly protonated caffeine (*m/z* = 195). With the ESI source, relative transmission was below 0.1% for all masses, compared to no FAIMS, and there were signs of incomplete desolvation.

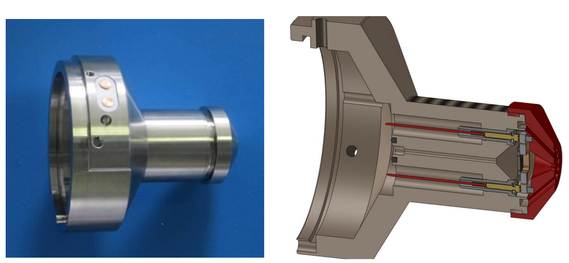

Figure 2. (a) Photo and (b) CAD drawing of first design iteration.

In the second design iteration, the chip was moved back towards the mass spectrometer inlet and thermal transfer cages were introduced to conduct more heat from the heated transfer capillary to the region upstream of the chip. The aim of these changes was to improve desolvation prior to the chip (Figure 3). Two versions of the chip cone, with different upstream bore diameters (0.8mm v 1.6mm), were made in order to explore the effect of upstream ion residence time on transmission and desolvation (Figure 4).

Figure 3. Second design iteration of microscale FAIMS chip. (a) Chip in the revised chip cone. (b) Chip interface fits over the spray cone of Thermo Scientific LTQ Orbitrap or Exactive series mass spectrometer. (c) FAIMS interface in place, with adaptor ring allowing attachment of any standard ionisation source. (d) Waveform feeder connector fits into adaptor ring to bring FAIMS waveform from controller module to chip.

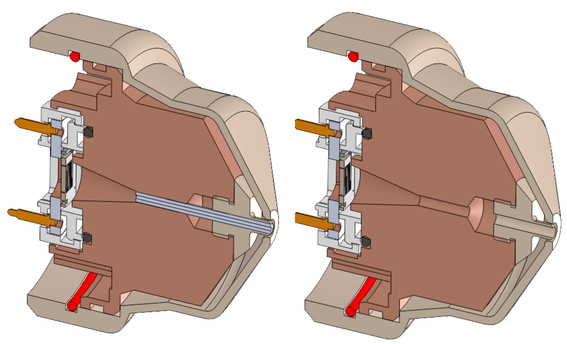

Figure 4. CAD drawings of (a) 0.8mm and (b) 1.6mm bore diameter chip cone

The narrower bore inlet (0.8mm) produced high transmission but unusually broad peak shapes (Figure 5a). This was attributed to uneven gas flow across the chip. Switching to the 1.6mm upstream bore restored normal peak widths, with some inevitable trade-off in ion transmission. This trade-off is expected as a wider bore results in slower gas flow in this region and hence higher diffusion losses.(Figure 5b).

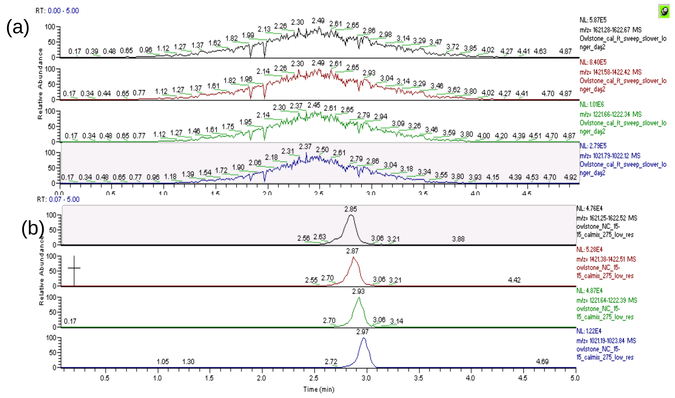

Figure 5. FAIMS spectra of ultramark 1621 oligomers at 295 Townsend (Td) dispersion field (DF) with (a) the narrow and (b) the wider bore diameter chip cone.

Transmission levels were assessed using an Ion Max ESI source. Results varied across the mass range, with relative transmission (ion abundance with FAIMS interface in place compared with no FAIMS) reaching 9% for *m/z* 609. This was a significant improvement on performance of the first design iteration.

4. Separation performance

The potential to fractionate samples prior to mass spectral detection was assessed by investigating the separation of multiple charge states of insulin. Partial separation of the 3+, 4+ and 5+ was observed (Figure 6).

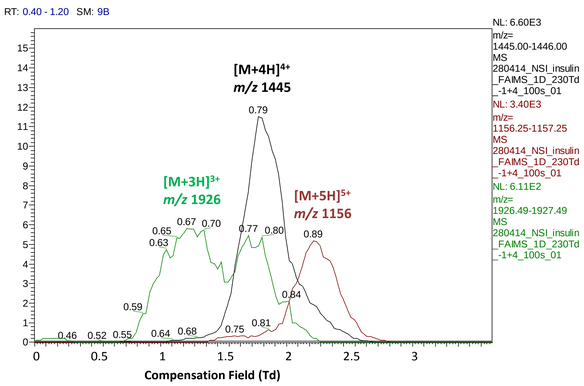

Figure 6. FAIMS spectra of insulin $[M+3H]^{3+}$ $[M+4H]^{4+}$ and $[M+5H]^{5+}$at 230 Td

Peak positions appeared to be influenced by charge, with higher charge states having a greater difference in mobility in the high and low fields. A double peak was observed for the $[M+3H]^{3+}$ ion, with the second peak coinciding with the peak from the $[M+4H]^{4+}$ species. Possible explanations may be the presence of multiple triply charged conformations or by the loss of a proton from a subset of $[M+4H]^{4+}$ ions between the FAIMS chip and mass spectrometer detector. Further investigation will be needed to understand this behaviour. FAIMS may provide a means of probing the interactions of different charge states in the atmospheric pressure region of an electrospray source.

References

[1] Swearingen KE, *et al*.. Mol. Cell Proteomics 11(4), M111.014985 (2012).
[2] Smith, R.W., *et al*., Journal of Chromatography A, 1278 (2013) 76– 81

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