Exploring the effects of carrier gas modifiers using chip-based field asymmetric waveform ion mobility spectrometry combined with mass spectrometry

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Introduction and Overview

- High-field asymmetric waveform ion mobility spectrometry (FAIMS) distinguishes ions at atmospheric pressure based on their differential mobility under low and high field conditions as the ions pass between two electrodes in a carrier gas
- The application of a compensation field (CF) allows transmission of selected ions of a selected differential mobility through the FAIMS device
- Chip-based FAIMS has been combined with TOF MS to allow rapid FAIMS pre-selection of gas-phase ions prior to mass analysis (FAIMS-MS)
- A novel solvent delivery system has been used to introduce organic vapour modifiers into the carrier gas flow
- The effect of aliphatic alcohol modifiers on the differential mobility and mass spectra has been explored for small molecules, peptides and proteins

Methods

Instrumentation:

- A miniaturized chip-based FAIMS device (Owlstone Ltd.) with a 100 μm electrode gap and 700 μm path length (Figure 1.a) was inserted in front of the capillary inlet of an orthogonal acceleration TOF MS (Agilent Technologies)
- The solvent vapour delivery system was constructed in-house (Loughborough University) to enable introduction of gas modifiers into the nitrogen carrier gas

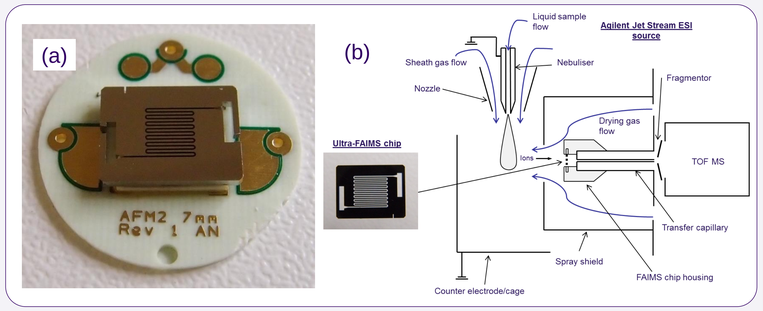

Figure 1. (a) Photograph of the miniaturised chip-based FAIMS and **(b)** Schematic diagram of FAIMS interfaced with the TOF-MS.

Experimental:

- Analytes including phthalic acids, peptides and proteins were directly infused (10 μL/min) into the ESI source, which was operated in negative ion mode for the phthalic acids and positive ion mode for proteins and peptides
- Peptides and proteins were diluted in methanol:water (50:50) + 0.1% formic acid to 10 pmol/μL
- Methanol and 2-butanol were added to the drying gas line (Figure 1.b) using a solvent vapour delivery system (100˚C); modifier concentration was application dependent in the range 0.25 to 2 % (v/v)
- ESI-FAIMS-MS conditions: sheath and drying gas flows, 9 and 7 L/min; sheath and drying gas temperatures, 250°C and 150°C; nebuliser pressure, 35 psig; spray shield, 2500 V; transfer capillary voltage and nozzle voltage, 3000 V and 2000 V respectively
- Dispersion fields (DF) of 200 - 300 Td were used in all experiments. Compensation Field (CF) scan range was analyte dependent.
- Fragmentor voltage was varied between 150 and 300 V. MS acquisition scan rate was 10 scans/s

Results: FAIMS-MS of phthalic acids

- Separation of isomeric *o*-, *m*- and *p*-phthalic acids (Figure 2) by mass spectrometry has not been reported
- The effect of methanol and 2-butanol modifiers was investigated over a range of concentrations and DF (200 to 300 Td)
- Addition of methanol vapour (Figure 3) shifts each phthalic acid peak differently, enabling *p*-phthalic acid to be selectively transmitted (Figure 3)
- Addition of 2-butanol changes the order of the phthalic acids in CF spectrum and the signal intensity varies significantly with 2-butanol concentration and DF field (Figure 4)
- Each phthalic acid isomer can be FAIMS-selected depending on choice of modifier, modifier concentration and dispersion field

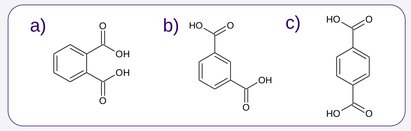

Figure 2. (a) o-phthalic acid; (b) m-phthalic acid; and (c) p-phthalic acid.

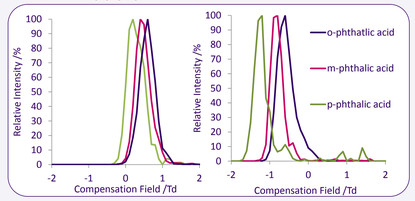

Figure 3. CF spectra of phthalic acids with **(a)** dry nitrogen and **(b)** 1% methanol; at DF 220 Td

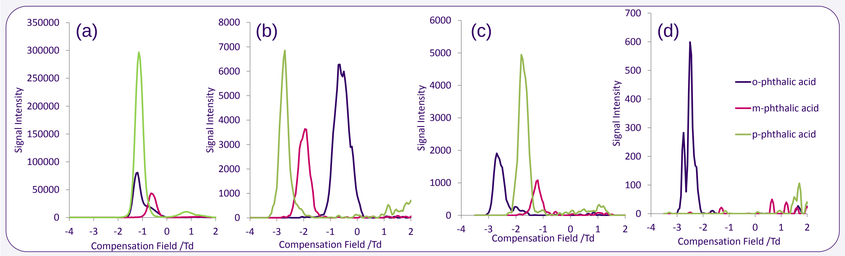

Figure 4. CF spectra of phthalic acids (SIR *m/z* 165) with 1% 2-butanol at **(a)** DF 210 Td; **(b)** DF 290 Td; and 2% 2-butanol at **(c)** DF 210 Td; **(d)** DF 290 Td

Results: FAIMS-MS of peptides

- The effect of 2-butanol on doubly charged bradykinin (Bk) and bombesin (Bo) was explored.
- Multiple conformers[1] of $[Bk+2H]^{2+}$ are observed with dry nitrogen (Figure 5.a), one conformer becomes favoured in the presence of 2-butanol (0.25%) giving a narrower peak (Figure 5.b)
- The intensity for $[Bk+2H]^{2+}$ at CF 1.2 Td is reduced, enabling the selective transmission of $[Bo+2H]^{2+}$ without interference from $[Bk+2H]^{2+}$ (Figure 5.i and ii)
- $[Bk+2H]^{2+}$ has a negative CF shift at higher levels of 2-butanol (data not shown)

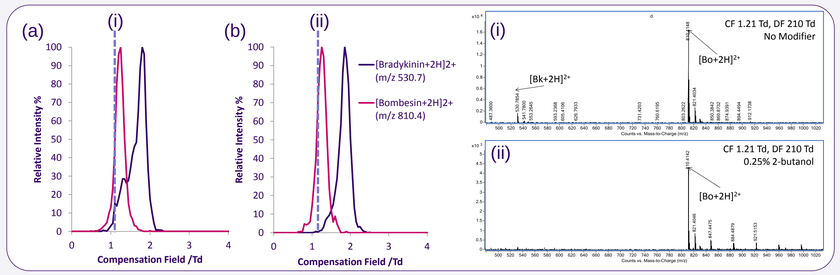

Figure 5. CF spectra of $[Bo+2H]^{2+}$ (SIR *m/z* 810) and $[Bl+2H]^{2+}$ (SIR *m/z* 530) at DF 210 Td with: **(a)** dry nitrogen; **(b)** 0.25% 2-butanol; and corresponding mass spectra taken from dashed line **(i)** dry nitrogen **(ii)** 0.25% 2-butanol.

Results: FAIMS-MS of proteins

- The effect of gas modifiers on proteins has not previously been explored, cytochrome c was selected as it is a commonly used model protein [4]
- The addition of methanol vapour changes the conformers of the +17 and +19 charge state of cytochrome c observed in FAIMS spectra but has no effect on the +12 charge state (Figure 6)

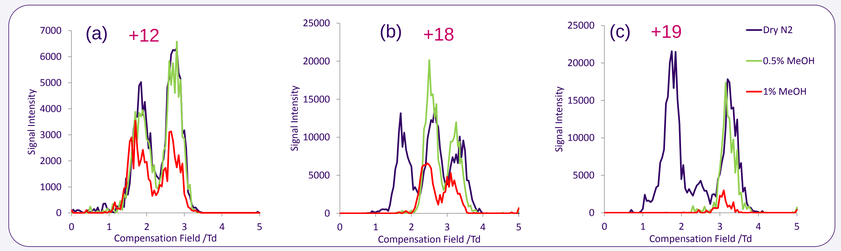

Figure 6. CF spectra of Cytochrome c: **(a)** +12 (*m/z* 1030.9), **(b)** +18 (*m/z* 687.6), **(c)** +19 (*m/z* 651.5) charge state at DF 300 Td with dry nitrogen, 0.5% methanol and 1% methanol

- The conformer peaks do not shift in the CF spectrum with a methanol modifier as observed for smaller molecules, but instead fewer conformations are favoured
- The +20, +21 and +22 charge states of cytochrome c were not observed in the MS (without FAIMS separation) with 1% methanol modifier, but were present in the spectrum without the modifier (Figure 7), suggesting that folded conformers are favoured in the presence of a modifier

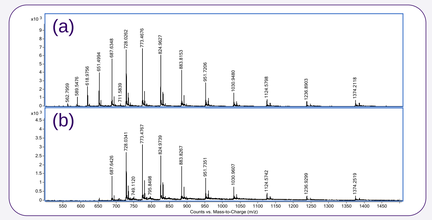

Figure 7. MS of Cytochrome C with **(a)** dry nitrogen, and **(b)** 1% methanol; without FAIMS separation

Conclusions

- Significant shifts in CF peak maxima were observed for small molecules with % level modifiers
- Positional isomers of phthalic acids could be selectively transmitted through the FAIMS device depending on the DF, choice of modifier and modifier concentration
- The CF spectra of peptides and proteins produced fewer peaks in the CF spectrum when alcohol modifiers were introduced into the carrier gas, showing a preference for folded conformers
- CF peak shifts with the addition of a modifier are greater for small molecules, where the difference between clustered and declustered ions is larger
- Separation, selectivity and signal intensity of analytes are influenced by the choice of modifier, the modifier concentration and the applied dispersion field

References

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