

The analysis of self-assembling supramolecular complexes of 3-methylxanthine using FAIMS and IMS combined with mass spectrometry

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Introduction

- Higher-ordered structures based on the self-assembly of simpler molecules are of interest in a variety of fields including structural biology, nanotechnology and supramolecular chemistry.
- Modified purine bases such as 3-methylxanthine (TMX) have been found to self-assemble in the presence of alkali metals and ammonium cations in the gas phase and in solution.
- Miniaturised high-field asymmetric waveform ion mobility spectrometry (FAIMS) and travelling wave drift tube ion mobility spectrometry (IMS), both combined with mass spectrometry, have been used to investigate self-assembling, non-covalent complexes of TMX in the gas phase.
- Travelling wave IMS (TWIMS) analysis has been used to determine collision cross sections (CCS) of selected TMX complexes.

Experimental

Sample Preparation

- 3-Methylxanthine was prepared as a 0.5 mM solution in 60:40 methanol:water with 1 mM ammonium acetate, or in 60:40 methanol:water with 1 mM sodium hydroxide, to promote the formation of TMX clusters with Na⁺, and to enable the detection of higher-ordered clustered TMX structures.

FAIMS-MS Parameters

- TMX solutions were analysed using an Agilent 6230 TOF (Agilent Technologies) with a modified Jet Stream ESI source, combined with a prototype miniaturised chip-based FAIMS device (Owlstone Ltd., Cambridge), located in front of the mass spectrometer inlet capillary (Fig. 1). The FAIMS device consists of multiple planar electrode channels each with a 100 μm gap and an electrode length of 700 μm.

- The MS experimental conditions in positive ion mode were: drying gas: 8 L/min at 150 °C; sheath gas: 10 L/min at 200 °C; nebuliser gas: 30 psig; capillary voltage: 3.5 kV; nozzle voltage: 2 kV; fragmentor voltage: 150 – 250 V; and a sample flow rate of 10 μL/min using a syringe pump. The optimum FAIMS conditions for the selective transmission of the different TMX clusters, singly, doubly and multiply charged species, were determined by conducting a compensation field (CF) sweep from -2 to 5 Td at a rate of 0.5 Td/sec, for dispersion fields (DF) in the range 194 to 323 Td.

IMS Parameters

- TMX solutions were analysed using a Waters Synapt HDMS spectrometer (Waters Corporation) fitted with a TWIMS drift cell, operated in IM-MS, IM-MS and IM-MS/MS modes. ESI conditions were capillary voltage: 3.0 kV; sampling cone: 20 L/hr; extraction cone: 4 L/hr; source temperature: 120 °C; desolvation temperature: 200 °C; desolvation gas flow: 400 L/hr. TWIMS analysis was performed with the travelling wave height at 7.5 – 12 V and 8 – 14 V with the N₂ drift gas set to 24 mL/min and the pusher interval set to 64 μs. The CCS of selected TMX clusters were determined using peptide compounds of known CCS measured in helium.

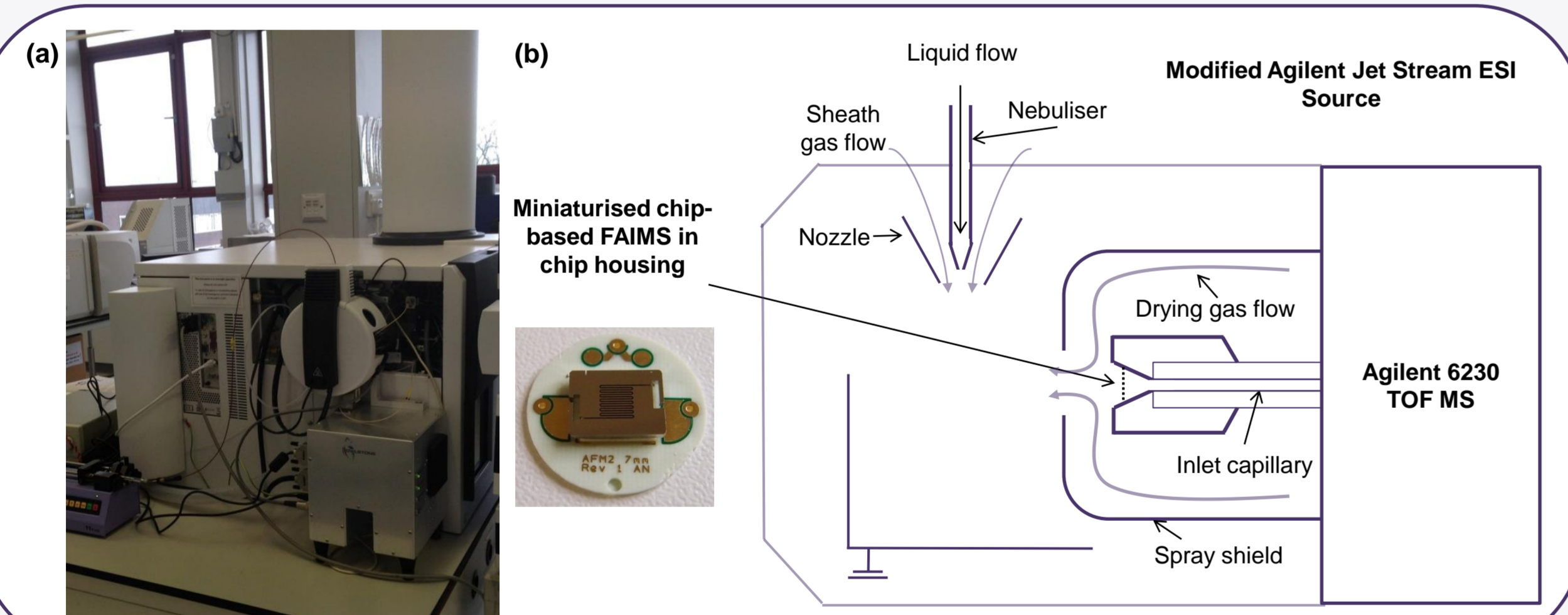


Figure 1: (a) Photograph of FAIMS-MS interface, (b) schematic diagram of the interface of the ion source region of the TOF-MS and the miniaturised chip-based FAIMS device

FAIMS-MS Results

- 3-Methylxanthine (TMX) is shown to self-assemble in the gas phase to form clusters around a stabilising cation (Fig. 2), which have been analysed using ESI-MS and ESI-FAIMS-MS. Supramolecular structures of TMX, where TMX forms tetrameric non-covalently bound structures around a NH₄⁺, Na⁺ and K⁺ cation have all been observed (Fig. 3). Clustering of TMX from single tetrameric complexes to higher-ordered quadruplex complexes of up to six TMX tetramers has been observed using FAIMS-MS in the presence of Na⁺ (Table 1). The focus of this preliminary study is on these monomeric and singly charged tetrameric complexes of TMX with sodium.

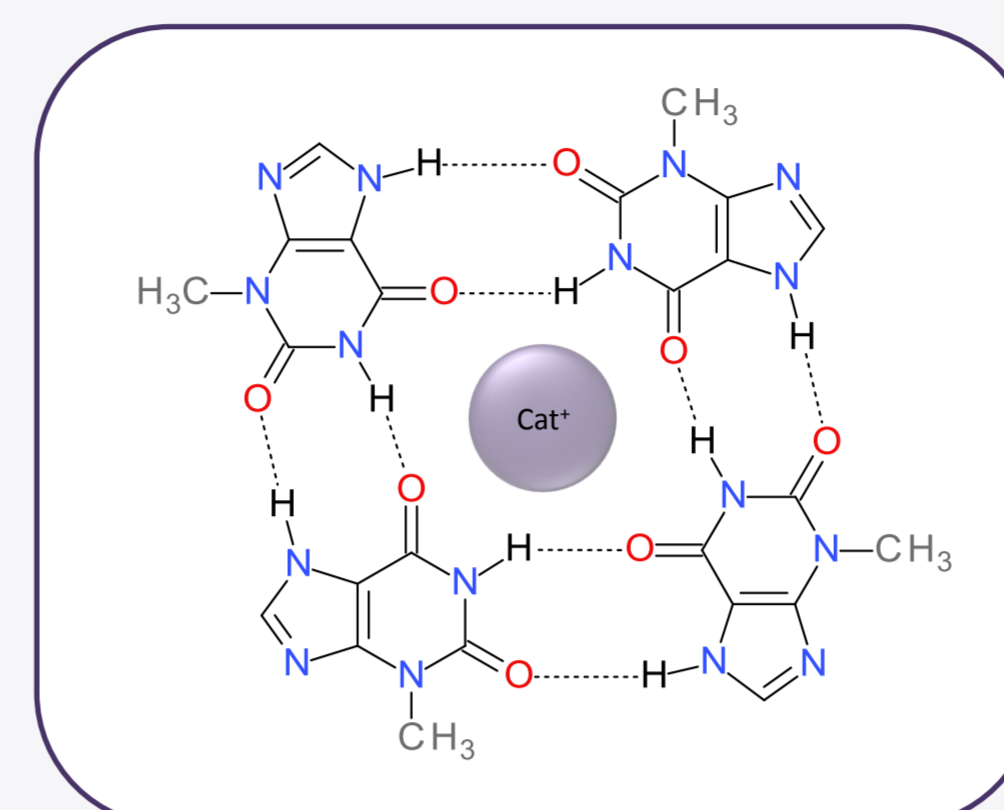


Figure 2: Structure of 3-methylxanthine non-covalently bound tetramer ([TMX4+Cat]⁺) with stabilising cation observed as NH₄⁺, Na⁺ or K⁺.

Table 1: TMX monomer and singly charged tetrameric complexes

TMX Complex	m/z
[TMX+Na] ⁺	189.04
[TMX4+Na] ⁺	687.18
[TMX8+Na] ⁺	1351.38
[TMX12+2Na-H] ⁺	2037.55

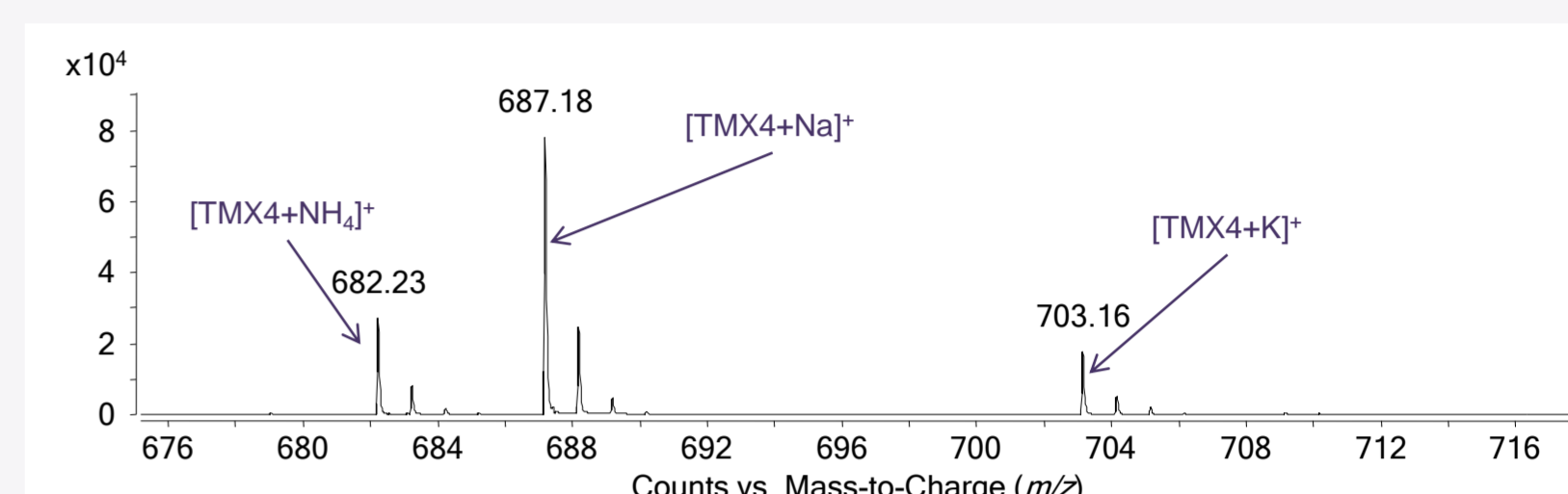


Figure 3: Mass spectra (no FAIMS) showing observed single tetrameric TMX species prepared in 1 mM

- FAIMS-MS has been used for the analysis of the non-covalent complexes formed by TMX. The singly charged TMX_n (n = 4-12) complexes show maximum FAIMS transmission at different CF values, with the optimum CF decreasing as the size of the cluster increases (Fig. 4).
- The signal to noise ratio of low abundance multiply charged species (Fig. 5) can be improved using FAIMS-selection prior to MS analysis.
- Separation of TMX complexes with different charge states can be achieved (Fig. 6) using FAIMS-selection of the appropriate charge state (Fig. 6 (d)).

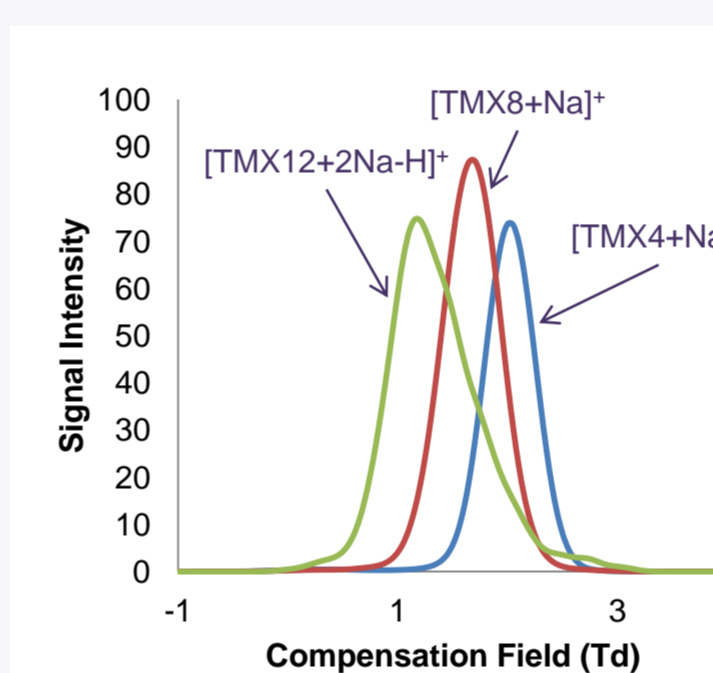


Figure 4: SIRs for a CF scan at DF = 323 Td for singly charged tetrameric TMX complexes

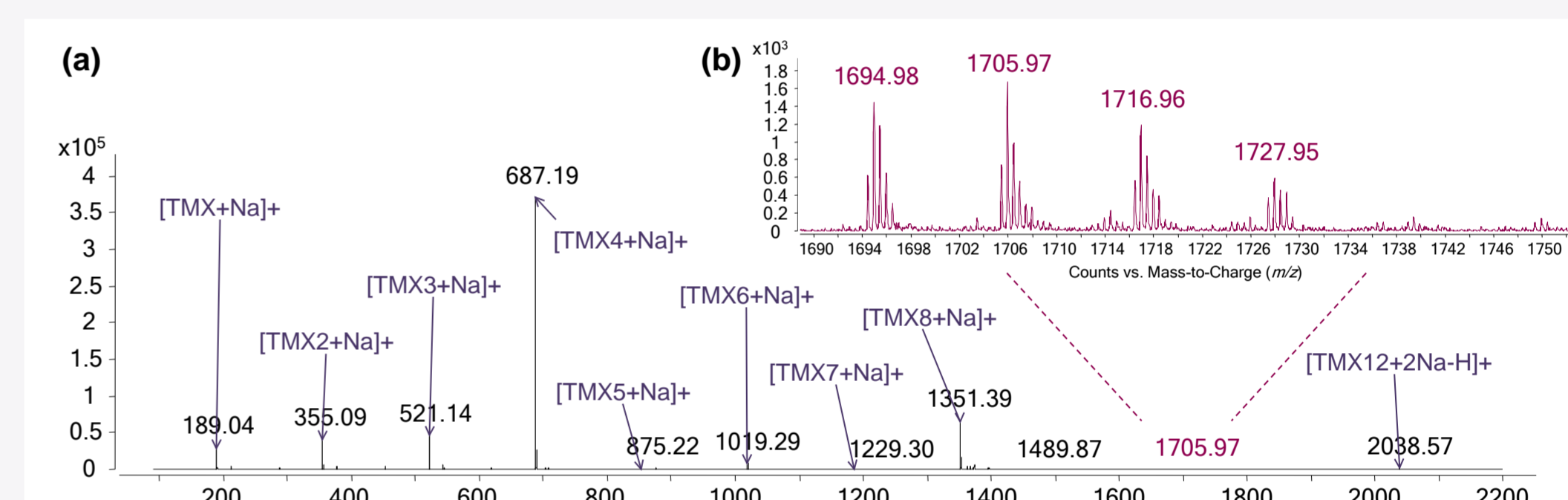


Figure 5: Mass spectra of TMX complexes (a) no FAIMS selection (singly charged TMX species labelled), (b) [insert] doubly charged TMX₂₀ species with Na⁺ with FAIMS-selection (DF = 259 Td, CF = 1.2 Td), which doubles the signal to noise ratio for these species

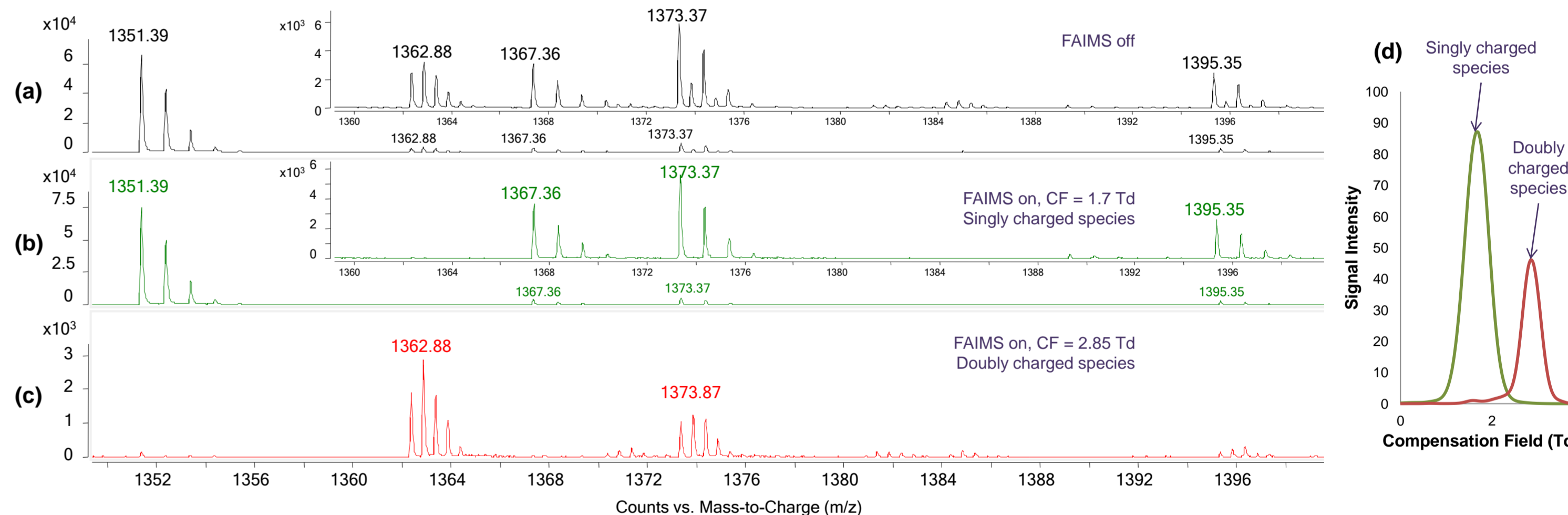


Figure 6: Separation of TMX (+Na⁺) complexes with different charge states using FAIMS (DF = 323 Td); (a) no FAIMS, (b) FAIMS-selection of the singly charged species (CF = 1.7 Td) and (c) FAIMS selection of the doubly charged species (CF = 2.85 Td); (d) FAIMS CF scan at DF = 323 Td (selected ion responses).

IMS-MS Results

- IMS-MS analysis of the sodium doped TMX complexes was used to determine the CCS of the singly charged tetrameric complexes.
- Experimentally measured CCS values (Fig. 7) were determined using peptide standards of known CCS (Table 2).

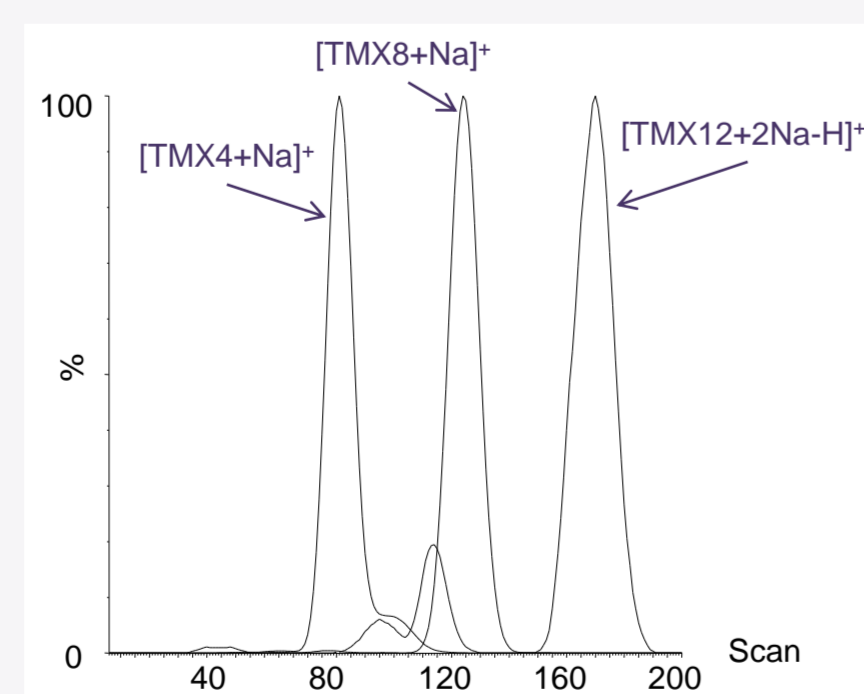


Figure 7: Ion mobility spectra of [TMX+Na]⁺ singly charged tetrameric complexes

Table 2: Experimentally measured CCS of [TMX+Na]⁺ singly charged tetrameric complexes

TMX complex	m/z	CCS (Å ²)
[TMX+Na] ⁺	189.1	80
[TMX4+Na] ⁺	687.2	181
[TMX8+Na] ⁺	1351.4	259
[TMX12+2Na-H] ⁺	2037.6	338

- IMS analysis combined with tandem MS (Fig. 8) allowed for further structural analysis of the fragmentation of these tetrameric TMX complexes in the gas phase.

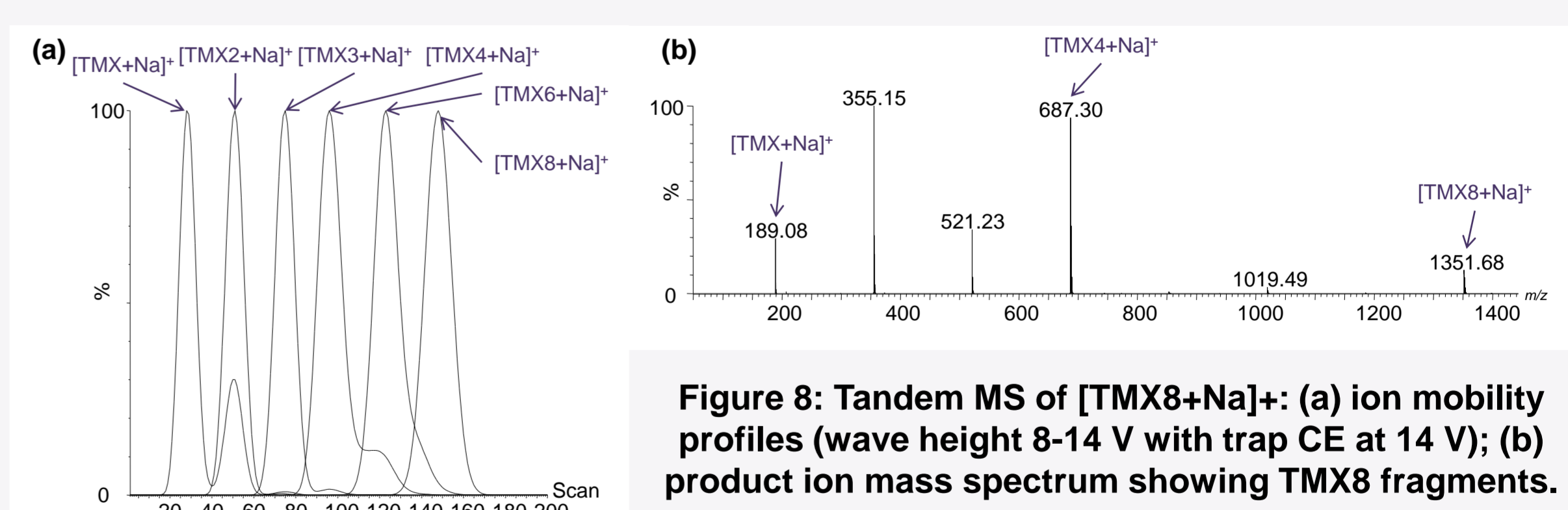


Figure 8: Tandem MS of [TMX8+Na]⁺: (a) ion mobility profiles (wave height 8-14 V with trap CE at 14 V); (b) product ion mass spectrum showing TMX₈ fragments.

Conclusions

- The hyphenation of FAIMS-MS and IMS-MS has been used for the analysis of TMX complexes.
- This preliminary study into the structural analysis of TMX complexes shows a complexity of non-covalently clustered structures.

- FAIMS-selection has been used for the separation of overlapping charge states of TMX complexes.
- Increased signal to noise ratio is observed for higher-order TMX complexes using FAIMS-MS.
- TMX singly charged complexes formed in the presence of sodium show different CF values for maximum ion transmission.

- TWIMS-MS analysis has been used to determine the CCS of selected singly charged TMX complexes.
- Tandem MS combined with IMS has been used to obtain ion mobility spectra of TMX fragments.

Acknowledgements

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