

An investigation of 3-methylxanthine supramolecular complexes using field asymmetric waveform and drift tube ion mobility spectrometry combined with mass spectrometry

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Introduction and Overview

- 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 chemistry¹.
- Modified purine bases such as 3-methylxanthine (3-MX) have been found to self-assemble in the presence of alkali metals and ammonium cations (Fig. 1) in the gas phase and in solution².
- Miniaturised high-field asymmetric waveform ion mobility spectrometry (FAIMS) and travelling wave drift tube ion mobility spectrometry (IMS), both combined with mass spectrometry (MS), have been used to investigate self-assembling, non-covalent complexes of 3-MX in the gas phase.
- Travelling wave IMS (TWIMS) analysis has been used to determine collision cross sections (CCS) of selected 3-MX complexes.

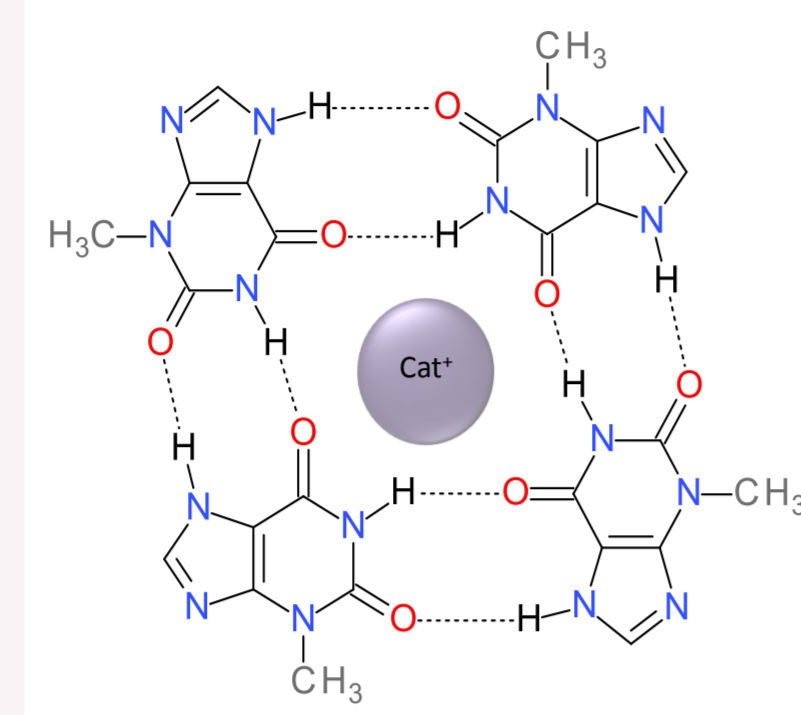


Figure 1: Structure of 3-methylxanthine (3-MX) non-covalently bound tetramer ($[(3\text{-MX})_4\text{+Cat}]^+$) with stabilising cation (NH_4^+ , Na^+ or K^+)

Methods

- 3-Methylxanthine was prepared as a 0.5 mM solution in 60:40 methanol:water with 1 mM ammonium acetate, or with 1 mM sodium hydroxide, to promote the formation of 3-MX clusters with Na^+ , and to enable the detection of higher-ordered clustered 3-MX structures.
- 3-MX solutions were analysed by FAIMS using an Agilent 6230 TOF MS (Agilent Technologies) with a 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. 2). The FAIMS device consists of multiple planar electrode channels each with a 100 μm gap and an electrode length of 700 μm .
- The TOF MS experimental conditions in positive ion mode were: drying gas: 8 L/min at 150 $^\circ\text{C}$; sheath gas: 10 L/min at 200 $^\circ\text{C}$; 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 $\mu\text{L}/\text{min}$ using a syringe pump. The optimum FAIMS conditions for the selective transmission of the different 3-MX 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.

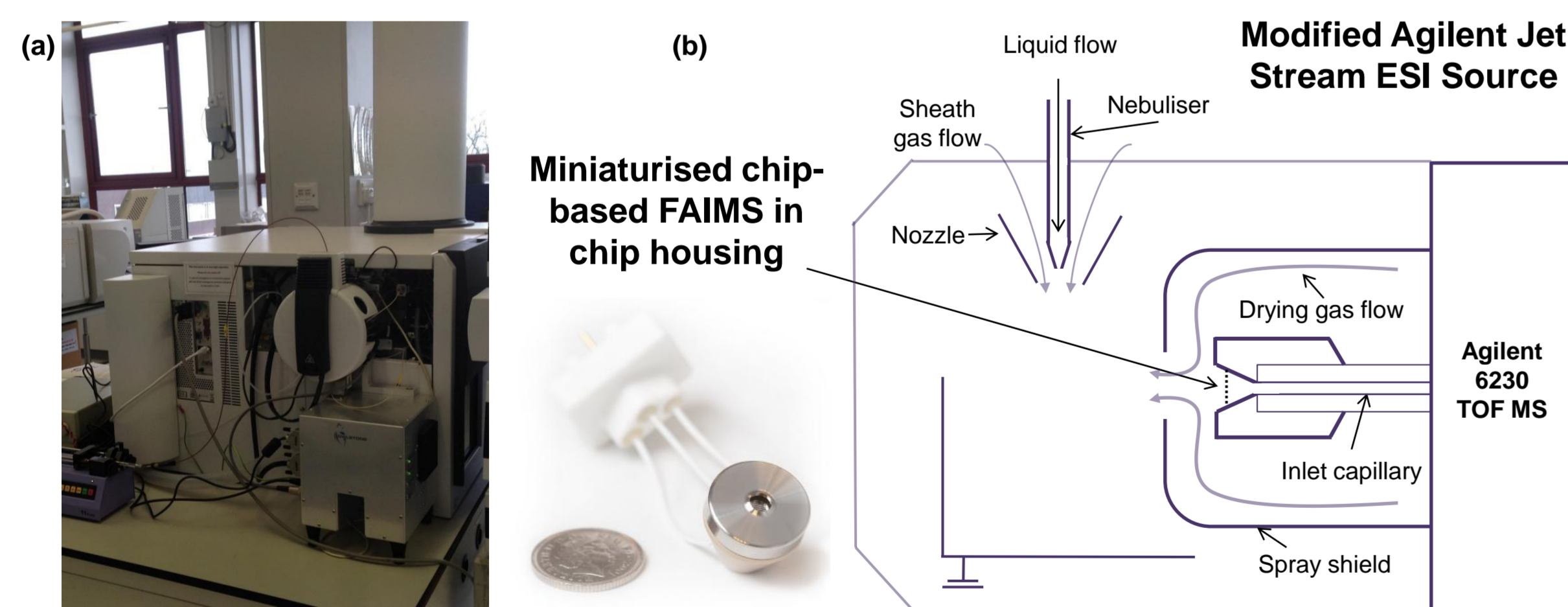


Figure 2: (a) Photograph of FAIMS-MS; (b) schematic diagram of the interface of the ion source of the TOF-MS and the chip-based FAIMS device

- 3-MX solutions were analysed by drift tube IMS using a Waters Synapt HDMS spectrometer (Waters Corporation) fitted with a TWIMS drift cell, operated in 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 $^\circ\text{C}$; desolvation temperature: 200 $^\circ\text{C}$; 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 N_2 drift gas set to 24 mL/min and the pusher interval set to 64 μs . The CCS of selected 3-MX clusters were determined using peptide compounds of known CCS measured in helium.

Results: FAIMS-MS

- 3-Methylxanthine (3-MX) is shown to self-assemble in the gas phase to form clusters around a stabilising cation (Fig. 1), which have been analysed using MS, FAIMS-MS and IM-MS.
- Supramolecular structures of 3-MX, where 3-MX forms tetrameric non-covalently bound structures around a NH_4^+ , Na^+ and K^+ cation have all been observed (Fig. 3) in agreement with Szolomájer et al².
- Clustering of 3-MX from single tetrameric complexes to higher-ordered quadruplex complexes of up to six 3-MX 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 3-MX with sodium.

Table 1: 3-MX monomer and singly charged tetrameric complexes

3-MX Complex	m/z
$[(3\text{-MX})+\text{Na}]^+$	189.04
$[(3\text{-MX})_4+\text{Na}]^+$	687.18
$[(3\text{-MX})_8+\text{Na}]^+$	1351.38
$[(3\text{-MX})_{12}+2\text{Na-H}]^+$	2037.55

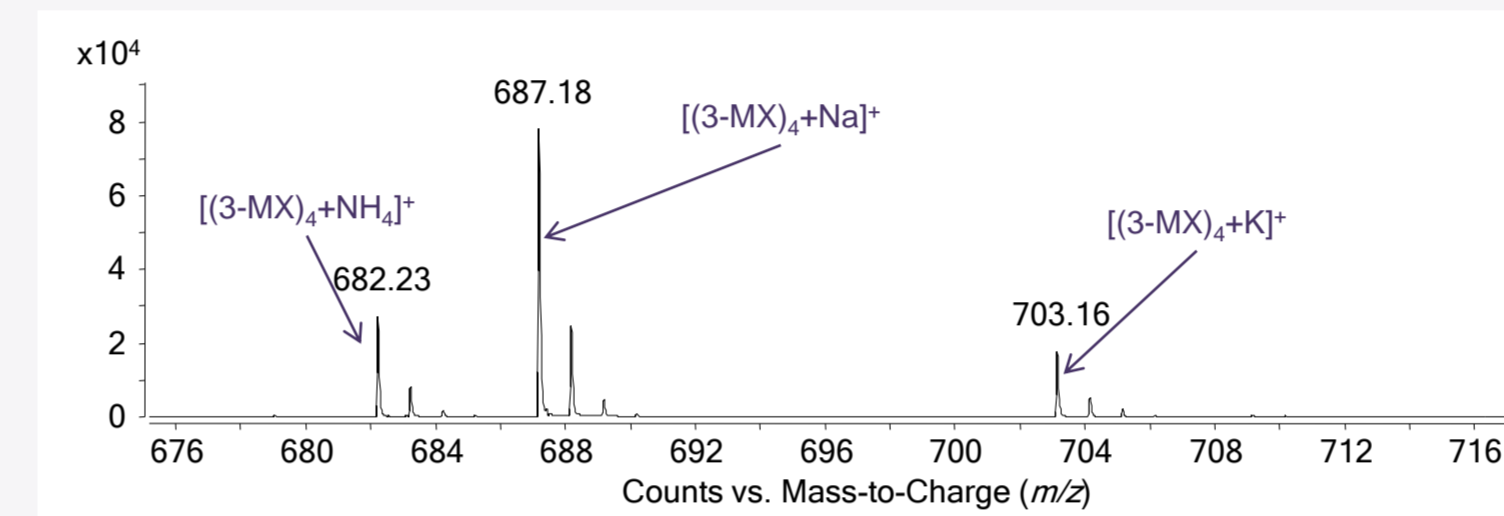


Figure 3: Mass spectra (no FAIMS) showing observed single tetrameric 3-MX species prepared in 1 mM sodium hydroxide

- FAIMS-MS has been used for the analysis of non-covalent complexes formed by 3-MX. The singly charged $(3\text{-MX})_n$ ($n = 4\text{--}12$) 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 (S:N) ratio of low abundance multiply charged species (Fig. 5) can be improved using FAIMS selection prior to MS analysis.
- Separation of 3-MX complexes with different charge states can be achieved (Fig. 6) using FAIMS selection of the appropriate charge state (Fig. 6 (d)).

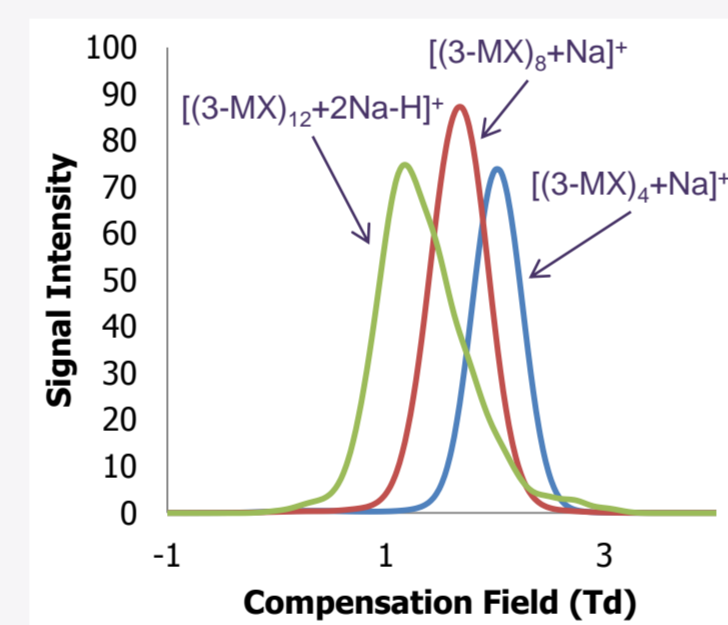


Figure 4: SIRs for a CF scan at DF = 323 Td for singly charged tetrameric 3-MX complexes

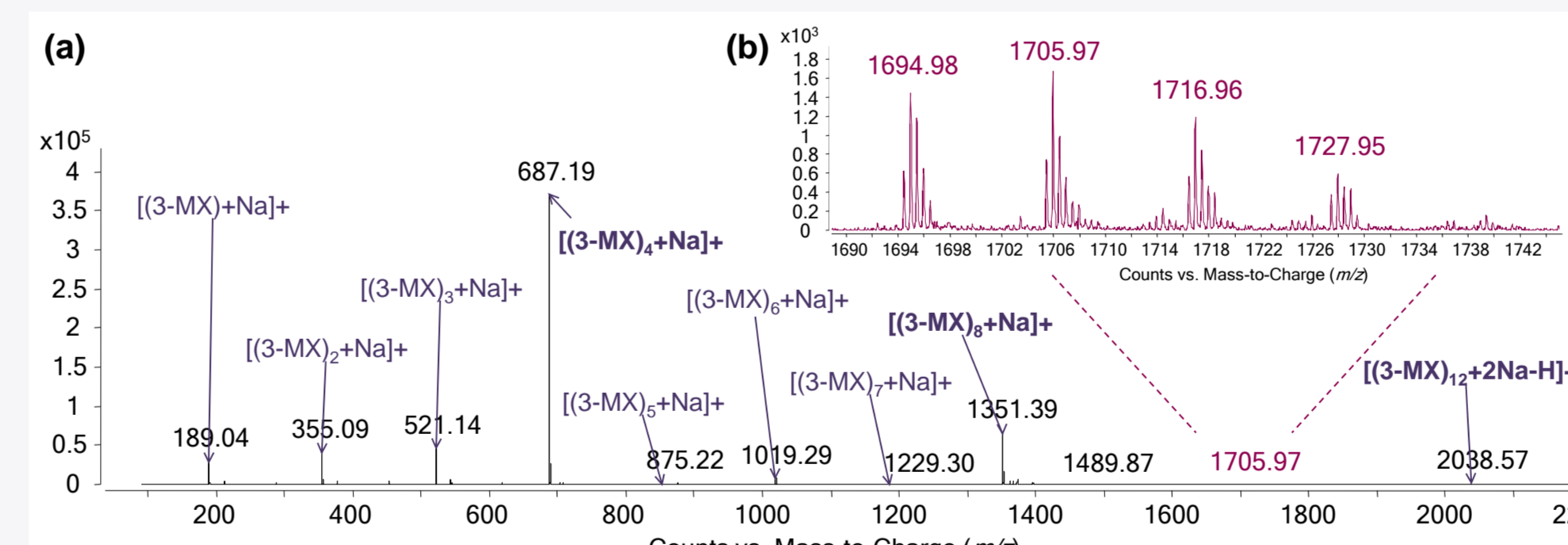


Figure 5: Mass spectra of 3-MX complexes (a) no FAIMS selection (singly charged 3-MX species labelled), (b) [insert] doubly charged $(3\text{-MX})_{20}$ species with Na^+ with FAIMS-selection (DF = 259 Td, CF = 1.2 Td), which doubles the S:N ratio for these species

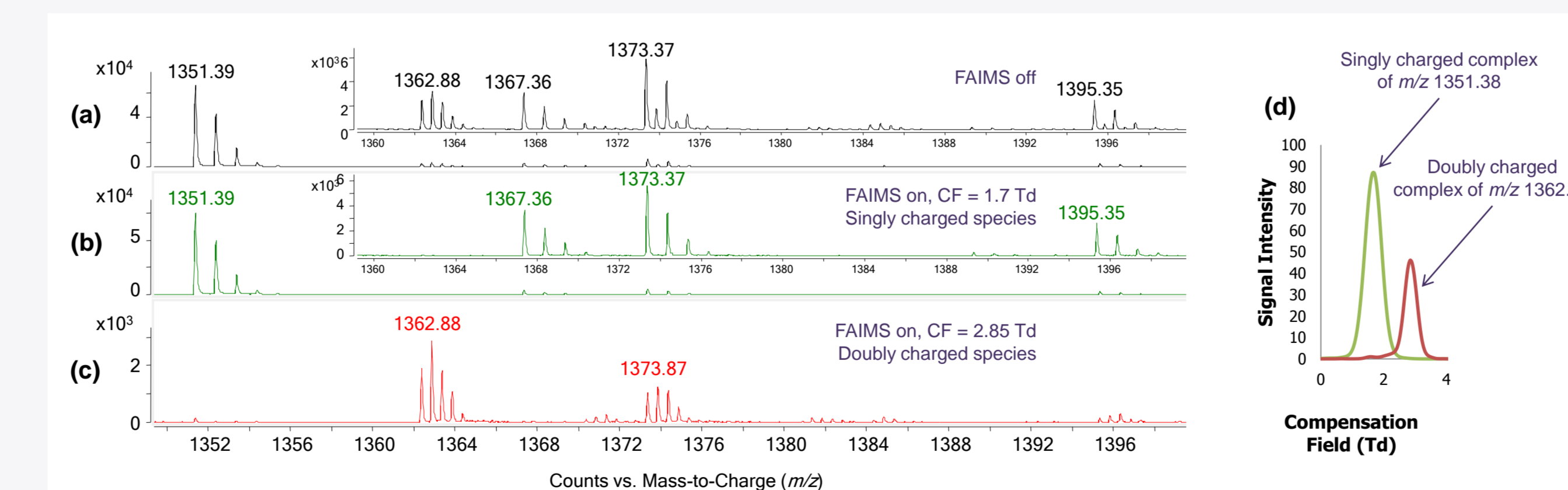


Figure 6: Separation of 3-MX (+Na⁺) complexes with different charge states using FAIMS (DF = 323 Td); (a) no FAIMS, (b) FAIMS selection of the singly charged species (CF = 1.7 Td) and (c) FAIMS selection of the doubly charged species (CF = 2.85 Td); (d) FAIMS CF scan at DF = 323 Td (selected ion responses)

Results: IM-MS

- IM-MS analysis of the sodium doped 3-MX 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).
- Preliminary modelling of $[(3\text{-MX})_4+\text{Na}]^+$ indicates a CCS of 184 \AA^2 , in agreement with the experimental data.

Table 2: Experimentally measured CCS of $[(3\text{-MX})+\text{Na}]^+$ singly charged tetrameric complexes

3-MX Complex	m/z	CCS (\AA^2)	± 3 SD (\AA^2)
$[(3\text{-MX})+\text{Na}]^+$	189.04	79	3
$[(3\text{-MX})_4+\text{Na}]^+$	687.18	180	2
$[(3\text{-MX})_8+\text{Na}]^+$	1351.38	258	2
$[(3\text{-MX})_{12}+2\text{Na-H}]^+$	2037.55	338	2

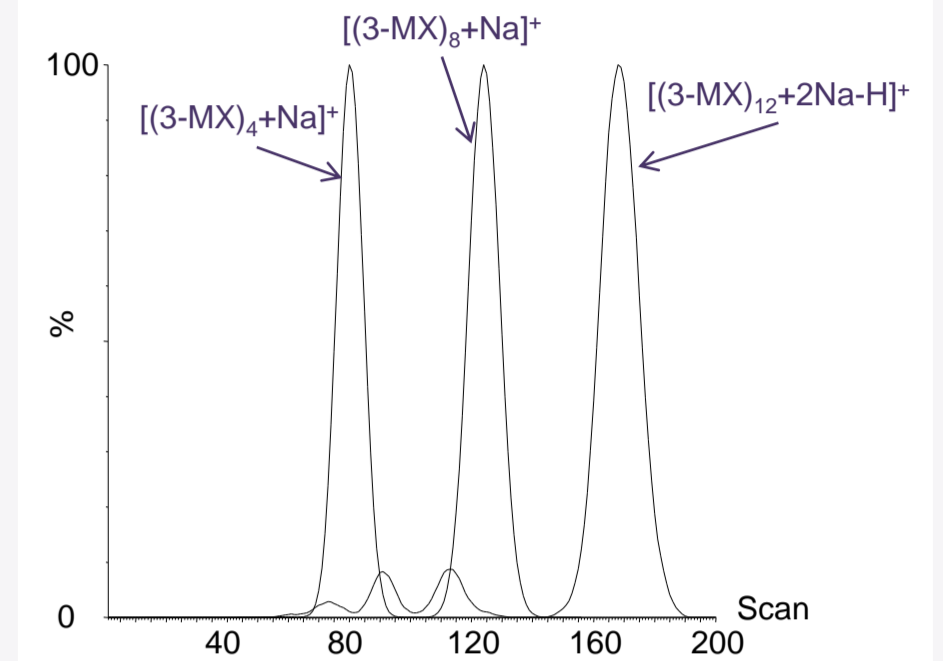


Figure 7: Ion mobility spectra of $[(3\text{-MX})_4+\text{Na}]^+$ singly charged tetrameric complexes

- IMS analysis combined with tandem MS (Fig. 8) allowed for further structural analysis of the fragmentation of these tetrameric 3-MX complexes in the gas phase.

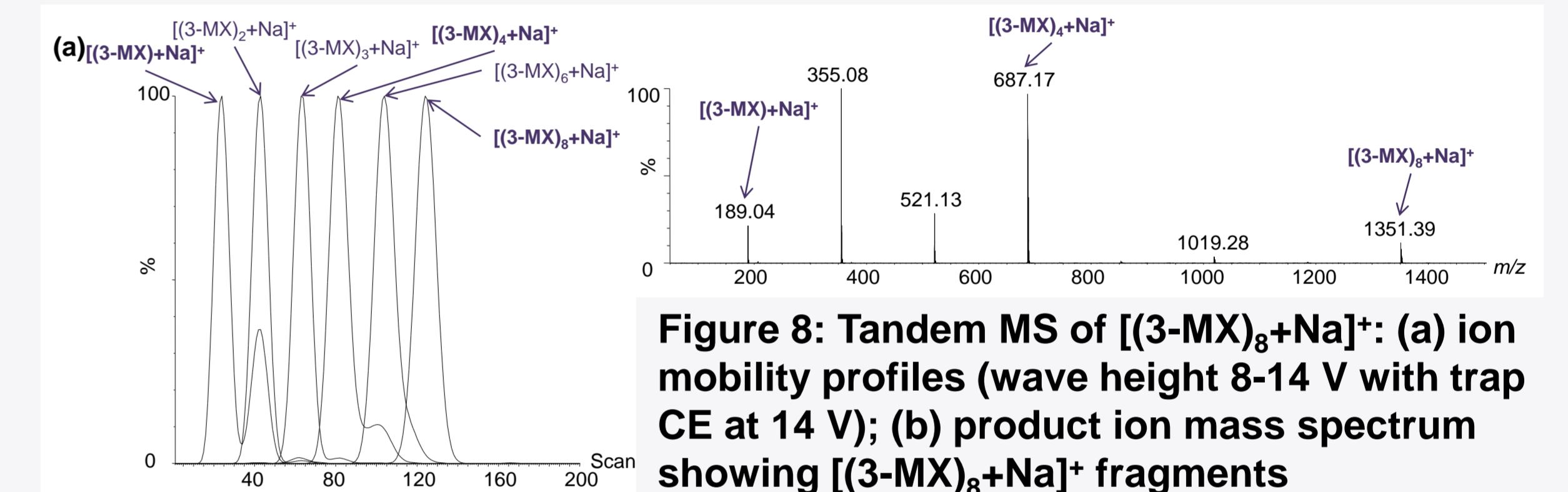


Figure 8: Tandem MS of $[(3\text{-MX})_8+\text{Na}]^+$: (a) ion mobility profiles (wave height 8-14 V with trap CE at 14 V); (b) product ion mass spectrum showing $[(3\text{-MX})_8+\text{Na}]^+$ fragments

Conclusions

- Hyphenation of FAIMS-MS and IM-MS has been used for the analysis of 3-MX complexes.
- This preliminary study into the structural analysis of 3-MX complexes shows a complexity of non-covalently clustered structures.
- FAIMS selection has been used for the separation of overlapping charge states of 3-MX complexes.
- Increased S:N ratio is observed for higher-order 3-MX complexes using FAIMS-MS.
- 3-MX 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 3-MX complexes.
- Tandem MS combined with IMS has been used to obtain ion mobility spectra of 3-MX fragments.

References

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