SPOTLIGHT feature

Mass Spectrometry

A Multi-Function Cyclic Ion Mobility - Mass Spectrometry System

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Interest in ion mobility (IM) separation coupled with mass spectrometry (MS) has grown considerably over the past couple of decades, finding utility in a broad range of application areas ranging from the study of small molecules to large protein complexes where either more peak capacity is required or determination of collision cross-section (CCS) values for analyte ion structural elucidation or confirmation is sought. Key to the resurgent development of IM-MS as an analytical technology has been the pioneering work of a number of academic research groups including Bowers (University of California Santa Barbera), Jarrold (Indiana University), Clemmer (Indiana University) and Hill (Washington State University) whose focus has been towards its application to the study of biological molecules. Another factor fuelling the growth in adoption of IM-MS has been the concomitant development and availability of instruments which provided both improved mobility resolution and higher transmission efficiency - the latter being pivotal to the analysis of biological samples at analytically relevant levels. The first high-performance commercial IM-MS system was the SYNAPT™ HDMS™ (Waters Corporation) quadrupole (Q)-IM-time-of-flight (ToF) instrument which was launched in 2006. This system provided high transmission efficiency in IM operation through use of ion accumulation and mobility separation in a radially-confining ion guide (minimising diffusive losses). This instrument also featured the first travelling wave (TW)-based mobility separator where, rather than the uniform, time invariant, electric field used in classical drift tubes, a repeating series of voltage pulses are continually passed through the device to provide mobility separation.

Over the ensuing years a number of iterations of the SYNAPT instrument, as well as introductions of commercial IM-MS instruments from other MS vendors, but the Q-IM-ToF arrangement remains unique to Waters and provides users with experimental flexibility to perform IM separation on either precursor and/or fragment ions. The desire to provide analysts and researchers with improved instrumentation, both in terms of raw performance and functionality, has rapidly driven the development of the technology. In 2019 Waters introduced the revolutionary SELECT SERIES™ Cyclic™ IMS providing a step change in the performance of commercially available instrumentation with both extremely high mobility resolution and the capability to do multiple stages of IM separation [1, 2].

A schematic of the Cyclic IMS instrument is shown in *Figure 1*(a). The instrument utilises the Q-IM-ToF geometry and, significantly, as the name suggests, the TW-IMS separator is cyclic rather than linear. The cyclic geometry enables ion mobility separation to occur over one or multiple passes around the device which provides 'dial-up' mobility resolving power (R) in a compact form.

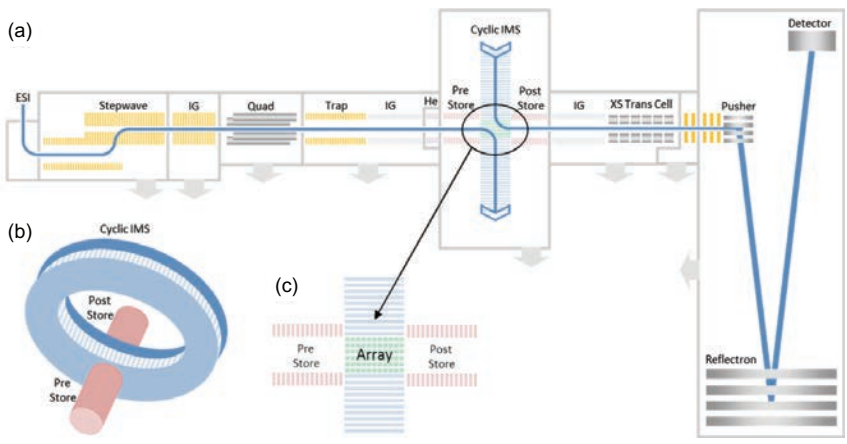

Figure 1. (a) Instrument schematic showing the Q-IM-ToF geometry. (b) Graphic showing the orthogonal arrangement of the Cyclic IMS and neighbouring ion optics. (c) Multi-function region (Reproduced with permission from ref [1]. Copyright 2019, Ujma et al.)

The cyclic IMS device has a 98 cm separation pathlength and is arranged orthogonally to the main ion optical axis of the mass spectrometer. It is operated at a pressure of ~2 mBar of N_2 and with travelling waves of up to 50 V amplitude and velocities generally in the 300 - 1000 m/s range. The enabling aspect of the design is the multifunction ion entry/separation/exit region of the IM device, consisting of an electrode array (*Figure 1*(c)). This region appears essentially identical to the main separation channel of the mobility device when separation is occurring (enabling multi-pass operation) but can switch function to facilitate the injection of ions into the device and ejection of ions from the device following mobility separation. The array can also be used to eject segments of the mobility separated ions to a pre-array store or a post-array store whilst the rest of the ions are removed, the stored ions are then re-admitted into the IM region for further separation. The stored ions can be activated, if required, on re-entry to the IM region to allow investigations of energy on the conformation of the ions or of the mobilities of fragment ions. The process of separation: isolation: activation: separation can be repeated many times, providing an IMS^n function in analogy with the MS^n capability of ion trap mass spectrometers.

The 'dial-up' resolving power capability of the cyclic IMS device is illustrated in *Figure 2* for the separation of two sodiated isomeric trisaccharides; Melezitose and Raffinose at m/z 527.2.

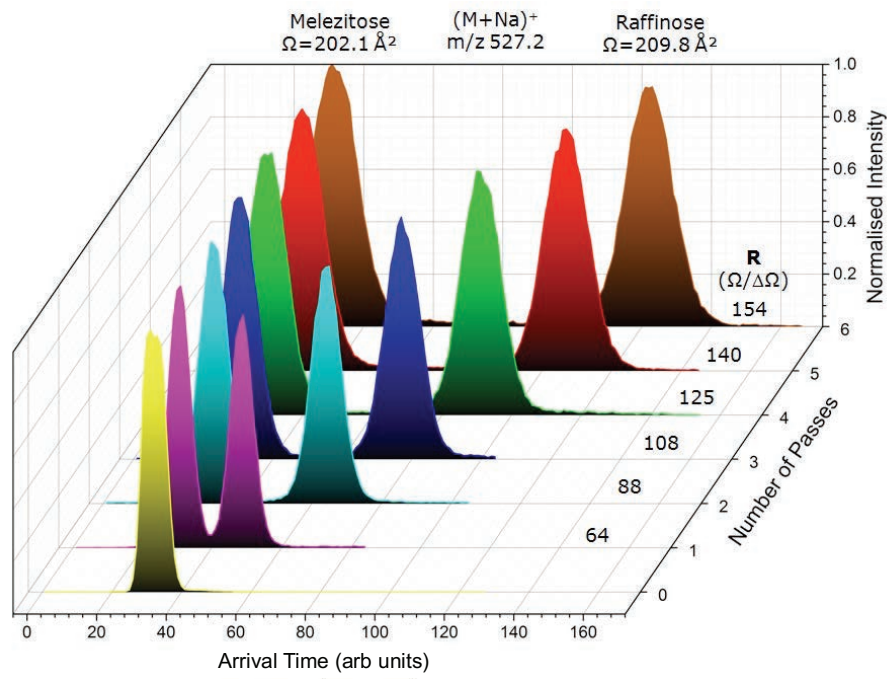

Figure 2. Cyclic IMS separation of two isomeric trisaccharides, Melezitose and Raffinose as a function of the number of passes (where R is the measured resolving power). The two species are separable because of their differing CCS (Ω) values.

It can be seen that R increases with increasing number of passes (n) around the cyclic IMS, and, as expected from theory, the resolution scales as √n. To date, the highest resolving power achieved on the cyclic IMS for singly charged ions is ~750 which

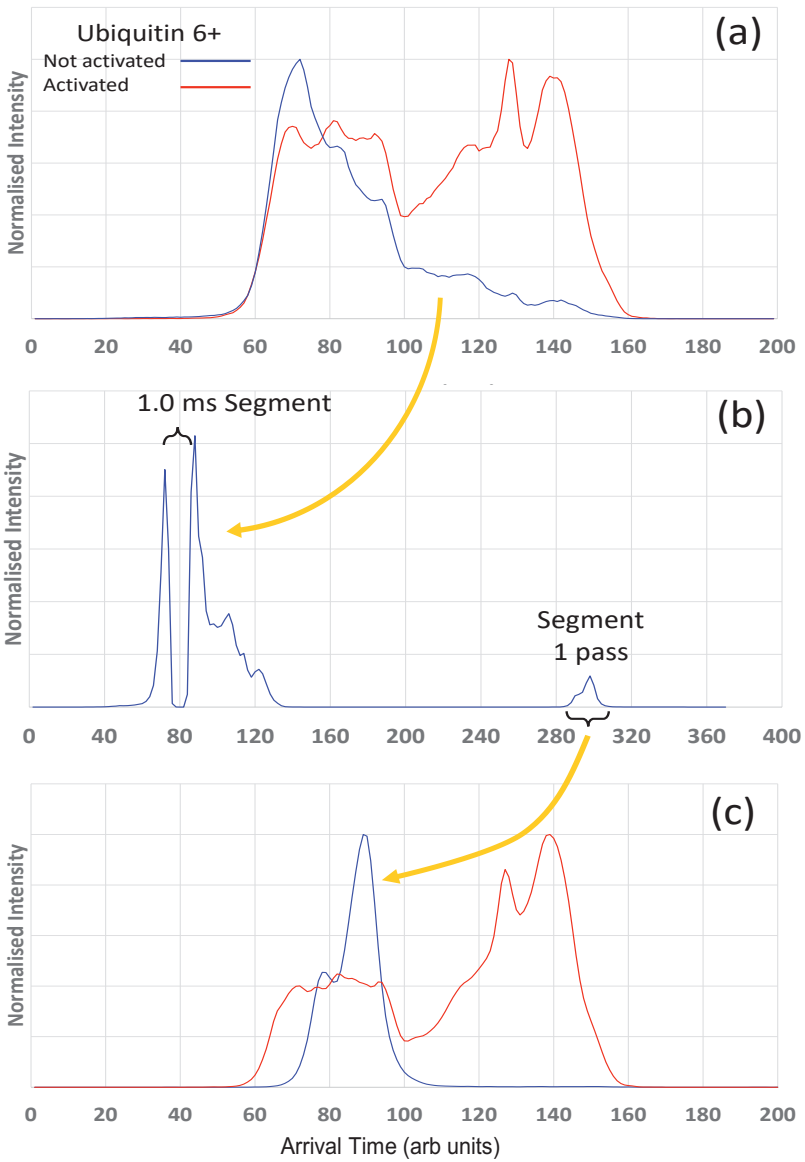

Figure 3. Arrival Time plots for Ubiquitin $(M+6H)^{6+}$ (a) single pass separation (b) 1 ms segment selected from single pass ions then a single pass on the selected ions (IMS^2) (c) expanded view of selected segment plot.

required 100 passes (98 m separation length) around the device [2].

The multifunction capability of the cyclic IMS is illustrated in *Figure 3*. Here the $(M+6H)^{6+}$ charge state of bovine ubiquitin has been m/z selected for mobility separation. *Figure 3*(a) shows the single pass separation for the non-activated ions (blue trace) and ions activated prior to entry into the mobility separator (red trace). From these data it is apparent that, even without activation, there is more than a single structure (conformation) of the (M+6H)6+ species present. Further, it can be seen that activation changes the distribution of conformations. *Figure 3*(b) shows the sequence of a single pass separation with a 1ms segment of the non-activated ions being ejected (using the array) to the pre-array store, followed by a single pass separation of these ions following re-injection to the cyclic IMS. *Figure 3*(c) shows the single pass separation of both the non–activated segment (blue, re-scaled) and the segment activated on entry to the cyclic IMS (red). Interestingly, the activated segment species has an arrival time profile close to that of the activated species in *Figure 3*(a) but originates only from a sub-section of precursor ions. This implies that on activation, essentially all conformations become accessible to the selected precursor ions, rather than a subset defined by their original conformation.

The advent of the cyclic IMS technology provides an incredibly powerful and flexible tool for in-depth sample analysis covering both fundamental and applied applications of a broad range of molecule sizes and classes. The illustrative examples presented here only hint at the capability of the overall IM-MS instrument. This present capability and ongoing developments ensure that the Cyclic IMS instrument will be at the forefront of the continued growth of IM-MS as an analytical technology for complex sample analysis.

References

1. Ujma, J.; Ropartz, D.; Giles, K.; Richardson, K.; Langridge, D.;Wildgoose, J.; Green, M.; Pringle, S., 'Cyclic Ion Mobility Mass Spectrometry Distinguishes

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2. Giles, K.; Ujma, J.; Wildgoose, J.; Pringle, S.; Richardson, K.; Langridge, D.; Green, M., 'A Cyclic Ion Mobility-Mass Spectrometry System' Anal. Chem. 2019, 91, 8564–8573