EXCELLIMS

Innovation for Excellence in Detection Technology

Enhanced Separation and Ion Prefiltering using a Modular High Performance Ion Mobility Interface for Mass Spectrometers

When coupled with mass spectrometry (MS), ion mobility spectrometry (IMS) adds tremendous value in the analysis of species that were either previously not resolved by MS or those that may lead to undesirable and highly complex spectra. As ion movement within a drift tube is governed by size/cross-sectional area, effective separation of molecules with slight structural differences such as conformers, isobars, and isomers can be achieved. In order to maximize the advantages of this technology, we have developed, evaluated, and introduce an ambient pressure IMS device (**Fig. 1**) that readily integrates with the ion trap product line of Thermo Scientific instrumentation.

Fig. 1 Excellims modular HPIMS add-on feature for mass spectrometers with API sources (shown with optional drift gas modification unit).

In recent years IMS-MS instruments have gained popularity in many institutions. Typically these integrated systems are home-built and quite customized (unfortunately limiting the widespread availability of such platforms), or are readily available commercially but rather cost prohibitive to many end users. In this work we present a new capability of interfacing a modular high performance ion mobility spectrometer (HPIMS) with mass spectrometers (i.e. Thermo LTQ Velos, Orbitrap) that are designed to accept an atmospheric pressure ionization (API) source, thereby eliminating the difficultly in constructing a customized IMS-MS system or the expense of a commercial version. Our device is available as a powerful add-on feature for various existing customer MS instruments already in current use. For only a fraction of the cost of a commercially available IMS-MS unit, our platform opens the door for simultaneous collection of additional valuable data and the opportunity to gain insight into the behavior of gas-phase ions not possible from mass spectra alone.

Presently our HPIMS interface is readily exchangeable with the Thermo Ion Max API source, requiring no additional hardware changes or modifications. Ions generated by either our Directspray™ or Infusion electrospray ionization (ESI) sources are sampled through an aperture into an “interface region” pumped by a diaphragm pump to assist in the initial desolvation of the ions (**Fig. 2**).

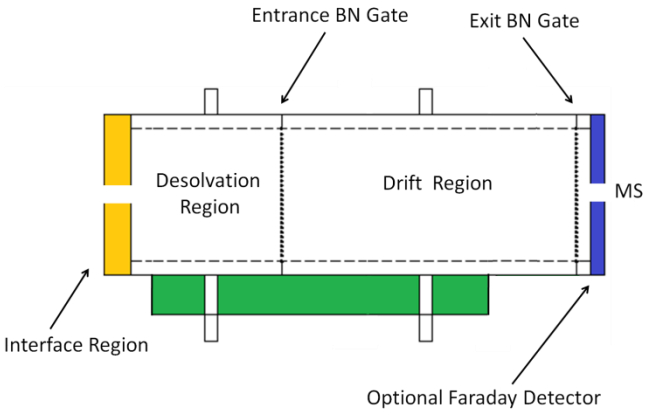

Fig. 2 Representation of the dual gate ion mobility module indicating interface region, desolvation region, ion gate #1, drift region, ion gate #2, optional Faraday plate detector, and ion trap interface. Coordinated dual BN gate system selects sections of drift time for entrance into the mass spectrometer.

Further desolvation is accomplished as ions are transferred through a second aperture into the "desolvation region" before being controllably introduced into the "drift region" by a pulsed Bradbury-Nielsen (BN) entrance ion gate. A second exit BN gate is located directly at the end of the 10.85 cm drift tube, effectively allowing only a user-defined portion of separated ions to pass. When open, the potential applied to adjacent wires of the ion gates was equal to that of the reference potential, a portion of the upper potential (8000 V) applied to the desolvation region. The gates were closed by applying a potential difference of ~60-70 V between wire sets. Embedded within Thermo's Tune Plus control software package is the ability to simultaneously control and pass ions into the MS with our dual gate IMS module in four distinct modes. In "open" mode, ions flow continuously through both ion gates unrestricted into the mass spectrometer. Although our IMS interface can rapidly be removed for reinstallation of the original API source, conventional mass spectral data can be obtained operating in this mode without the risk of a significant reduction in ion transmission efficiency. Second, the selected ion mobility can be scanned, sequentially stepping a window of variable drift width across the chosen drift time range ("scan" mode). This mode is of particular importance in the analysis of species with unknown drift times, or additionally if the user wishes to generate a comprehensive multi-dimensional IMS-MS plot from a complex mixture. Third, mass spectra can be collected at individually specified ion mobilities ("gated" mode), which allows ions to be selectively accumulated on the basis of their mobility prior to MS analysis. Several unique features are highlighted for this mode of operation including:

(A) Removal or *prefiltering* of contaminants and spectral interferences
(B) Targeted ion accumulation for *lower detection limits* and *enhanced MS^n fragmentation*
(C) Greatly *improving sensitivity* due to the inherent advantage of extended ion accumulation times possible with trap style instrumentation
(D) Isolating and examining *conformational states*

Lastly, the selection and passage of multiple ion mobilities concurrently is made possible when operating in "notched" mode. In this manner, all of the benefits afforded by the "gated" mode are possible with the additional advantage of enabling complete transmission for a series of ions (i.e. protein charge state envelope). Injection gate widths, gating voltages, scan rate, scan averaging, and drift ranges can be adjusted and optimized within the Tune Plus control software for maximum resolution and sensitivity.

For an additional level of advanced method development, we also offer a drift gas modification unit (DGMU) to promote improved mobility separation using structure selective interactions between analytes and specific compounds infused into the drift gas. This mechanism of gas-phase separation with our DGMU has been shown to separate chiral molecules (R,S) and (S,R)-ephedrine and (S,S) and (R,R)-pseudoephedrine which contain an internal hydroxyl group at the first chiral center and an amino group at the other chiral center utilizing n-octanol as a drift gas modifier.[1]

Our IMS platform further affords the ability to function as an independent, stand-alone instrument with the optional Faraday plate detector accessory. Mounting directly behind the second BN gate, our detector facilitates the investigator to perform IMS analyses with the module installed or even detached from the mass spectrometer. This feature greatly improves laboratory efficiency as IMS experiments (not requiring subsequent MS functionality) can be performed while another user is able to have full access to the mass spectrometer. The required high voltage electronics to operate our drift tube, ion gates, and optional Faraday detector are conveniently supplied in a supplemental control box.

The investigation of samples containing pseudo-isobaric/isobaric species demonstrates one of the many advantages of IMS separation prior to MS detection. Samples of octabenzone and PEG400 were ionized and transferred into the mass spectrometer with the IMS module operating in "open" mode. As shown in **Fig. 3**, both protonated octabenzone and one of the PEG400 ions ($C_{14}H_{30}O_8 + H^+$) are observed at an m/z of 327. Without IMS, these ions would otherwise be difficult to

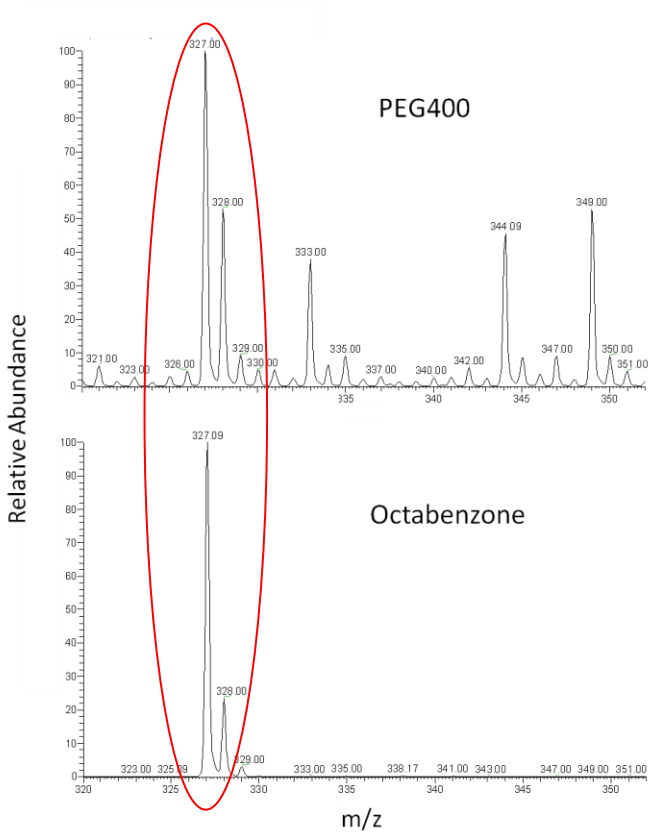

Fig. 3 Individual mass spectra of PEG 400 and octabenzone, both components present at m/z 327.

distinguish by mass spectrometry alone. However, simultaneously ionizing octabenzone/PEG400 and scanning the injection waveform over a drift range of 30 ms yields two distinct peaks (m/z: 327); one centered at 16.4 ms (PEG400) and another at 18.2 ms (octabenzone).

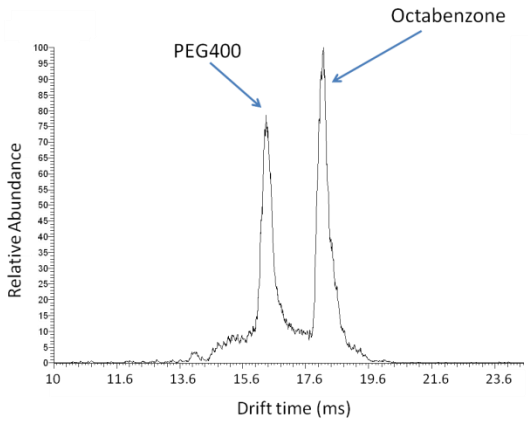

Fig. 4 IMS separation of PEG400/octabenzone prior to injection into the mass spectrometer.

The identities of these peaks were confirmed by (A) performing collision-induced dissociation (CID) of the selected precursor ion while only sampling a narrow window of drift times around each peak, and (B) running the ion trap in selected ion monitoring (SIM) mode and subsequently dissociating the selected ion while re-scanning the injection waveform. Further experiments were carried out with additional pseudo-isobaric/isobaric compounds and the merit of pre-MS separation with our IMS interface is exhibited in **Fig. 5**.

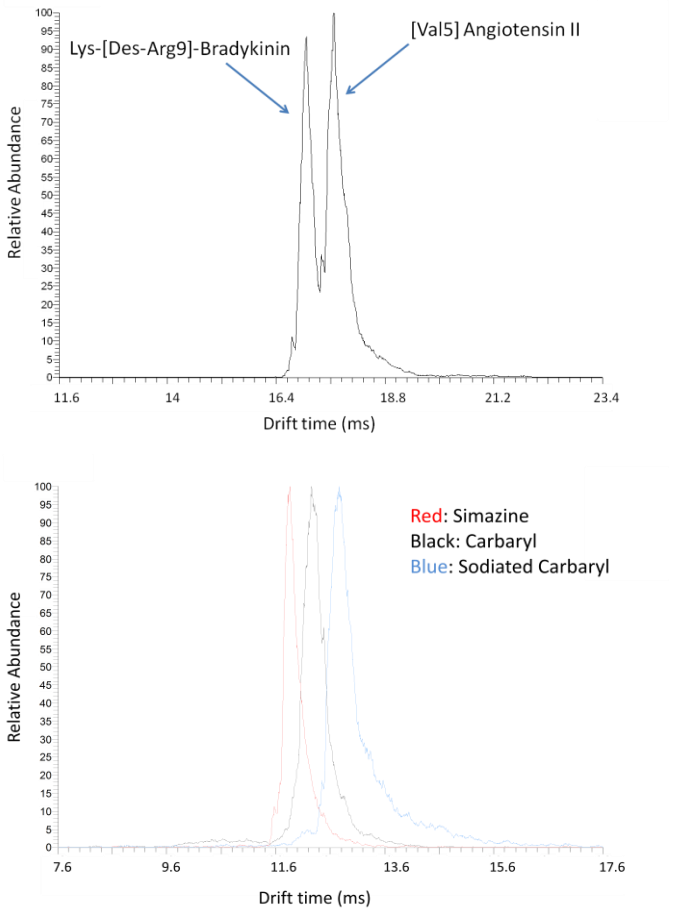

Fig. 5 IMS "scan" mode for (A) Lys-[Des-Arg9]-Bradykinin + [Val5] Angiotensin II (m/z: 517) and (B) simazine + carbaryl (m/z: 202). Note: separation of sodium or similar adducts can result in improved quantitative accuracy.

In addition to demonstrating the effective separation of pseudo-isobaric species, the separation of PEG400 and octabenzone substantiates the significance of upstream

IMS for the removal of polymer peaks from the desired analyte. Polyethylene glycols (PEGs) are commonly used in biological and pharmaceutical analyses to stabilize proteins for separation, purification, delivery, and storage.[2] Furthermore, PEG is a widely observed contamination arising from various organic solvents and detergents. When present in samples intended for MS analysis, ionization of sample components is rather challenging due to the heightened ion signal suppression arising from PEGs. Cleanup methods can be implemented to remove salts, detergents, and other interferences, however, such purification procedures are generally time consuming, low throughput, and risk the loss of precious sample. Although both desired and undesired species may still be ionized and introduced in our HPIMS-MS interface, the ion trap can be selectively filled, considerably decreasing the spectral congestion.

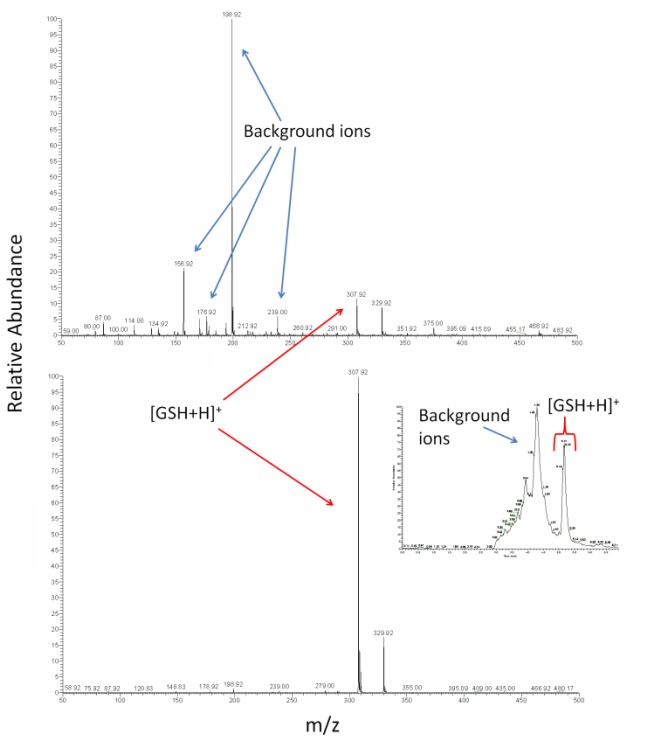

Fig. 6 Presence of unwanted background ions co-populating trap with GSH (top), and mass spectrum following removal of background ions by operating in "gated" mode, only allowing $[GSH+H]^+$ to accumulate in the ion trap (bottom). [Inset displays initial IMS scan used to identify drift times of background ions for exclusion].

An additional example of contaminant ion elimination for enhanced mass spectra using our IMS device is illustrated in **Fig. 6**. The existence of several intense background ions are observed when no IMS filtering is applied (top spectrum). However, with IMS acting as a pre-filter, a considerably cleaner mass spectrum (bottom spectrum) is the result of removing these interferences, allowing the previously low abundance peak ($[GSH+H]^+$) to become the main peak observed. The ability for removal of a variety of contaminants encountered in mass spectrometry can have broad implications in many applications, as a large number of such interferences have been commonly reported in the literature.[3] As trap technology advances towards high capacity devices, we envision mass spectrometers equipped with our IMS module to experience appreciably cleaner mass spectra achieving lower sensitivity for ions that were previously hindered by matrix.

It should be noted that our device is capable of attaining these separations with high resolution. As depicted in **Fig. 7**, a resolution over 100 is possible in the separation of two peptides (GSH and GRGDS). Routinely operating with a resolving power of over 60, there exists the potential for the IMS-MS system described herein to accomplish the required separation and detection found when utilizing LC-MS. Unlike LC-MS, HPIMS-MS would involve a profoundly simplified method and rapid screening.

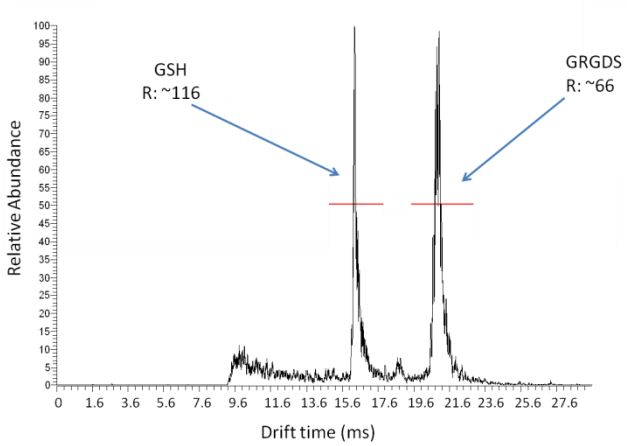

Fig. 7 High resolution separation of GSH and GRGDS, indicating potential of peptide/protein digest analysis when MS system combined with our IMS module.

Showing promise for the analysis of biomolecules, we are able to readily separate peptide and protein charge states (**Fig. 8**). The phenomenon of multiply charging proteins during the electrospray ionization process has important implications and advantages when considering performing tandem mass spectrometry (MS/MS) experiments. Upon fragmentation, more fragment ions are observed with multiply charged precursor ions than with singly charged precursor ions. Specifically electron transfer dissociation (ETD) requires more than one charge on the molecule and is most effective with highly charged cations.[4-7] The capability of selectively accumulating protein ions of a specific charge state (with more charges) in the ion trap would promote a markedly improved occurrence of dissociated ions during MS/MS.

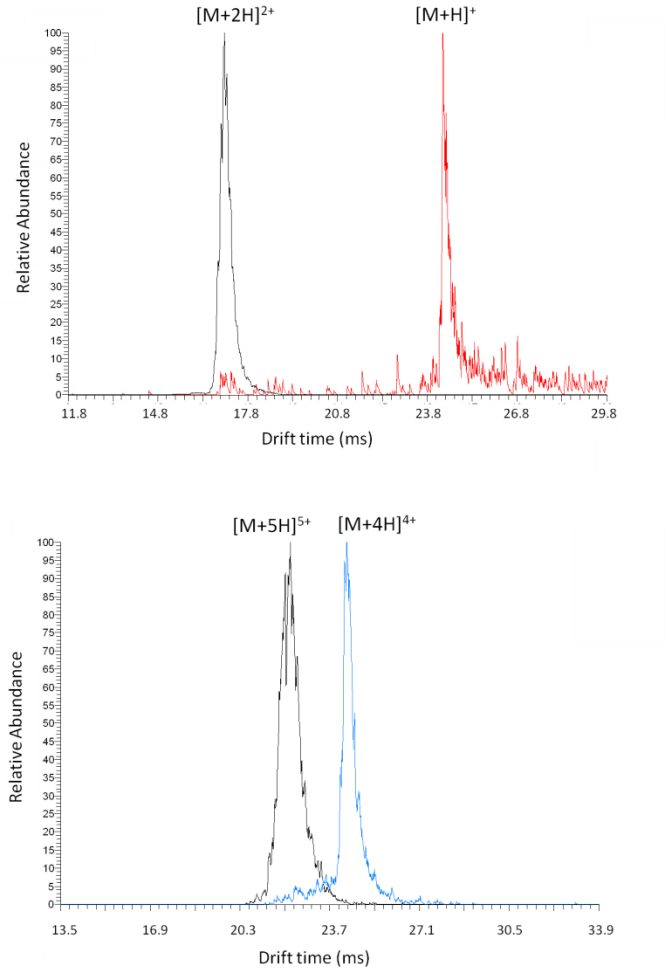

Fig. 8 (Top) Separation of +1 and +2 bradykinin, (bottom) separation of +5 and +6 insulin.

There has also been considerable effort to reduce the spectral complexity in ESI mass spectra of protein mixtures, as each component can generate multiple ions of distinct m/z ratio.[8] Partially overlapping distributions from multiple proteins would no longer serve problematic, as individual charge state envelopes could be analyzed by MS.

Several proteins commonly studied due to the presence of co-populated conformational states (e.g. cytochrome c in **Fig. 9**), have been investigated. As supported by the presence of a number of distinct species associated with various charge states (as shown on our multi-dimensional m/z versus drift time versus intensity plots), the IMS module provides the unique opportunity to gain insight into protein systems where the difference in structure is highly relevant for specific functionality as well as misfolding leading to diseased states.

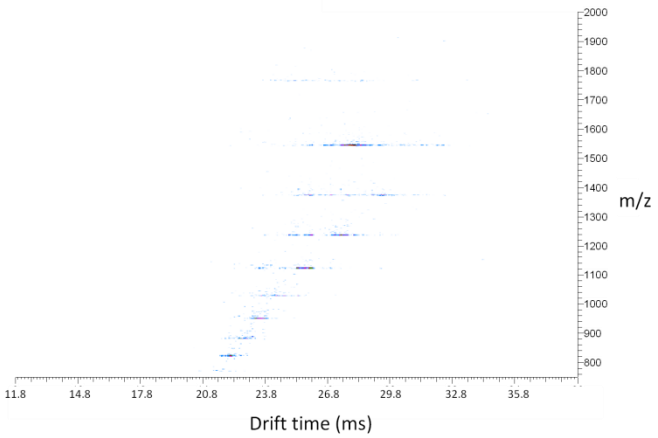

Fig. 9 Multi-dimensional IMS-MS plot of cytochrome c made possible with our device.

References

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