[WHITE PAPER]

TRIWAVE

More Complete Characterization of Mixtures and Molecules

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INTRODUCTION

This white paper describes:

- Benefits of T-Wave™ Ion Mobility Separations (IMS)
- Triwave design and implementation of T-Wave IMS

The continued need for more comprehensive characterization of complex mixtures or complex molecules in today's analytical applications is a primary driver for the development of powerful new mass spectrometry technologies. With higher levels of separation power (selectivity) and new experimental capabilities (versatility), scientists from a wide range of disciplines can carry out more complete profiling of mixtures and structural characterization.

Based on 'Travelling wave'[1,2] (T-Wave™ is a type of stacked ring ion guide, also referred to as T-Wave ion guide, or TWIG) technology, Triwave® is a unique device designed to maximize sample separation in an analytical workflow using ion mobility separations (IMS).[3,4] Conventional Mass Spectrometry enables separation of ions based on *m/z*, the addition of IMS further enables the differentiation of ions by size, shape and charge, as well as *m/z*. Triwave therefore provides an additional, orthogonal dimension of separation in a short timescale enabling scientists to:

- Increase analytical peak capacity with an extra orthogonal dimension of separation power.
- Ability to identify and quantify with more coverage and confidence.
- Derive information on conformation and structure with mass spectrometry.
- Deliver the most comprehensive structural characterization with novel fragmentation routines.

T-Wave IMS benefits

There are a number of benefits to using T-Wave ion mobility that have been reported in over 200 peer-reviewed papers.[5]

Orthogonal separation power – The second generation Triwave device has an IMS resolution sufficient to separate:

- Isobaric ions, ions of very similar *m/z* but different compounds.
- Conformers or isomers that have multiple species with subtle changes in size and shape, as shown in Figure 1.
- Compounds of different classes.
- Ions of different charge state.[3]

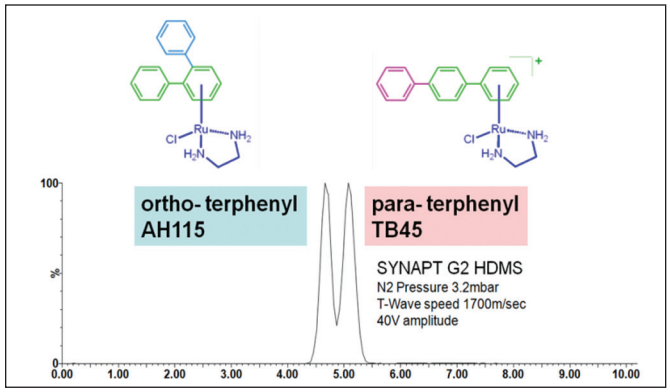

Figure 1. Separating small molecule drug isomers[3] with T-Wave ion mobility.

" It is shown that IMMS reveals 2 to 3 gas-phase conformer populations for IgG2s. In contrast, a single gas phase conformer is revealed using IMS for both an IgG1 antibody and a Cys-232 Ser mutant IgG2, both of which are homogeneous with respect to disulfide bonding. This provides strong evidence that the observed IgG2 gas-phase conformers are related to disulfide bond heterogeneity. Additionally, IMS MS analysis of redox enriched disulfide isoforms allows unambiguous assignment of the mobility peaks to known disulfide structures. These data clearly illustrate how IMMS can be used to quickly provide information on the higher order structure of antibody therapeutics."

BAGAL D., *ET AL.*
Rapid Commun. Mass Spectrom. 2008; 22: 2898-2904.

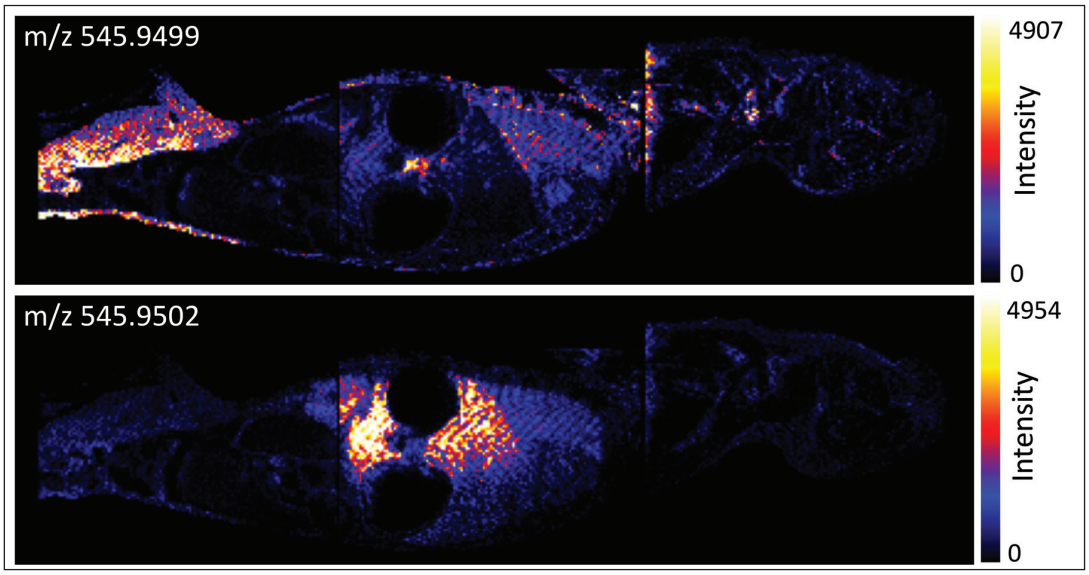

Figure 2. Visualization of the distributions of two ions (shown in red in the m/z versus drift time plot), which differ significantly in drift time but have a mass difference so small that it would require a m/z resolving power in excess of 1.8 million to achieve differentiation of the ions without T-Wave IMS separation. The spatial distributions of the two ions are clearly different, when plotted using m/z and drift time to generate the images, as shown in the two panels for m/z 545.9499 and m/z 545.9502.

Improved coverage and confidence

Due to the compatibility of analytical timescales, shown in Figure 3, T-Wave IMS can be employed in hyphenated approaches, without detriment to duty cycle or data quality.

This can provide significant gains in selectivity (analytical peak capacity, shown in Figure 4) and specificity (confidence in results, shown in Figure 5), when compared to the use of *m/z* separation alone:

- Up to 100% increase in identifications compared to non-IMS based approaches.[7]
- Improved LOD and LOQ where new detections are typically at the lowest concentrations.[6,7]
- Improved confidence in identification or confirmation with cleaner, interference-free fragment ion spectra.[7]

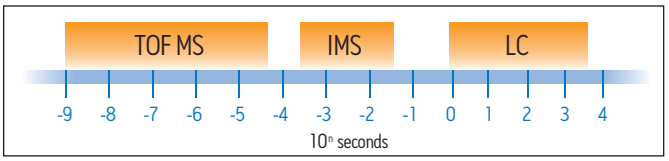

Figure 3. Compatible timescales – IMS separations integrate perfectly between those of UPLC and Tof.

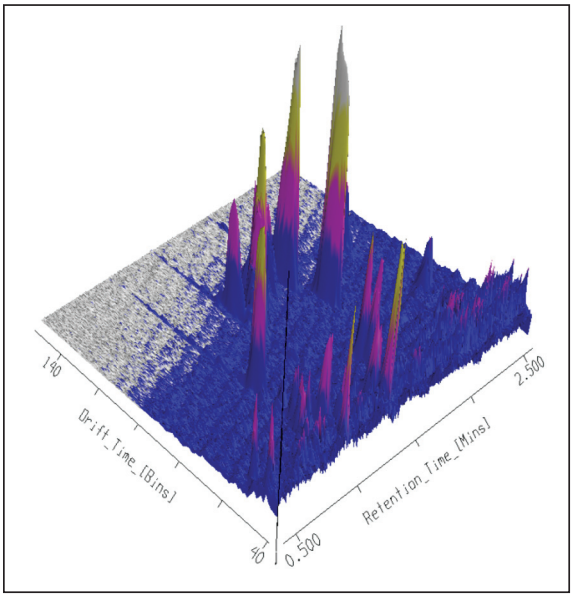

Figure 4. A plot of retention time versus drift time, illustrating how peak capacity can be increased by embedding T-Wave IMS with UPLC/MS.

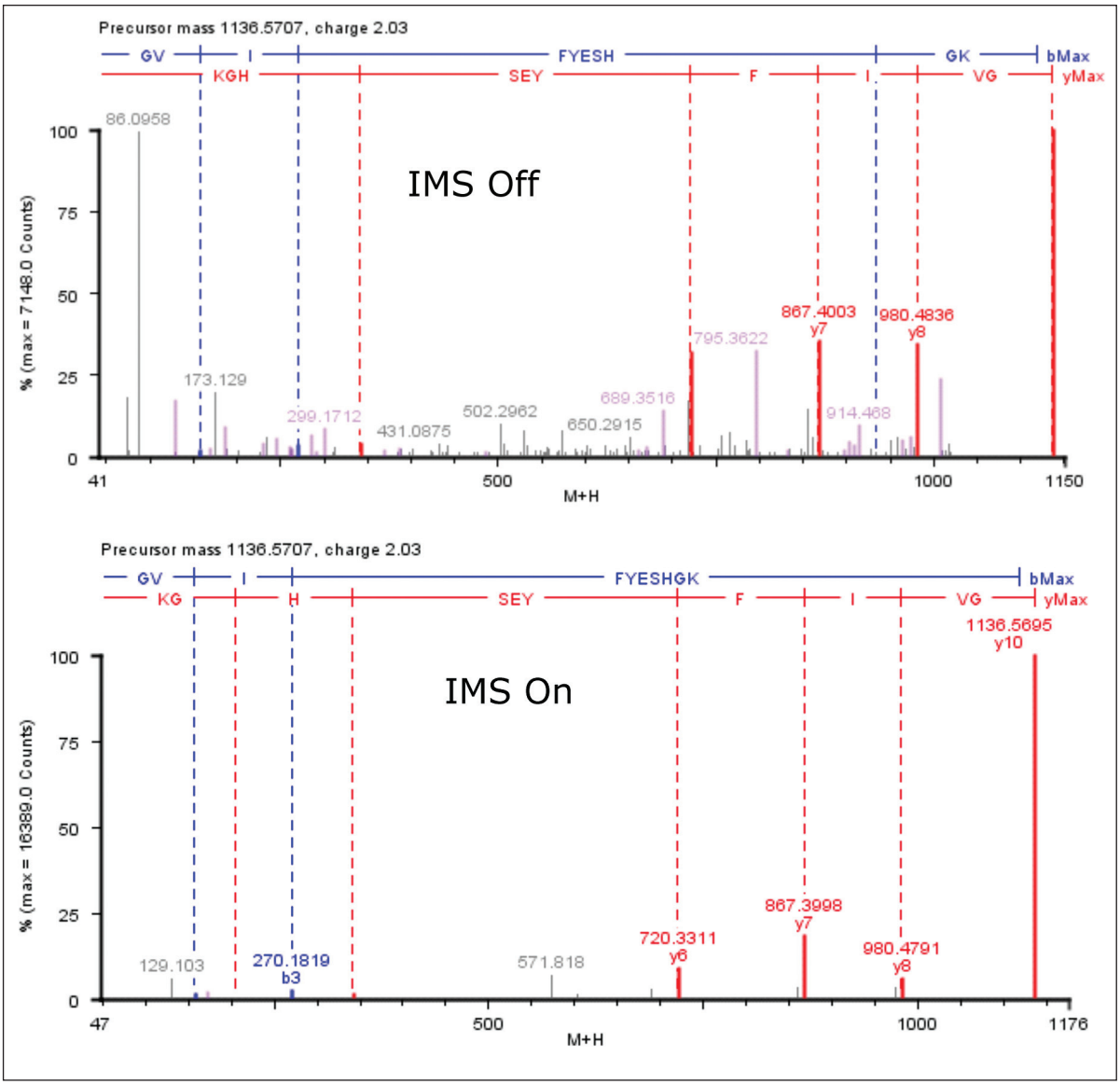

Figure 5. Generating cleaner spectra for more confident detection and identification from complex mixtures (above spectrum shows identified peptide from a protein digest mixture). Analysis with IMS eliminates significantly reduces interference (grey peaks in upper spectrum) from ions of similar m/z or rt, to those of interest.

"The Waters SYNAPT G2 with ion mobility has greatly enhanced selectivity for proteomic analysis. An orthogonal measure, ion mobility dramatically improves the label-free MS^E methodology used in our research."

ANDREW K. OTTENS, Ph.D.
Assistant Professor of Anatomy & Neurobiology and Biochemistry, Virginia Commonwealth University, VA, USA

Providing information on conformation

T-Wave IMS enables the characterization of molecular structure through the determination of collision cross section values (CCS), which is a measure of the average rotational collision cross section of a molecule. T-Wave IMS can be used as a powerful complementary approach to traditional structural methods[8] (X-ray crystallography, Nuclear Magnetic Resonance spectroscopy, or Electron Microscopy) through its ability to:

- Separate isomers, isoforms, and isobaric molecules from mixtures.
- Provide conformational measurements at very low (physiological) concentrations.
- Enable analysis of compounds over a very wide *m/z* range (20 to 100,000 *m/z*).
- Elucidate structural changes, *e.g.* biotransformation.[9,10]
- Provide novel research insights, with numerous publications[5] reporting the use of CCS values from protein complexes, to small molecules.

More detailed structural characterization

The geometry of Triwave Technology provides a range of options to increase information from fragmentation experiments, shown in Table 1.

These techniques have been applied successfully to a wide range of applications including, but not limited to:

- Structural elucidation of small molecules[10,11] and lipids[12,13,14] (TAP fragmentation).
- Top-down sequencing of proteins.
- Simultaneous confirmation of site of modification and peptide sequence with ETD or dual-stage CID.

TRAP T-Wave	IMS T-Wave	TRANFER T-Wave
-	IMS	-
CID or ETD	IMS	-
-	IMS	CID
CID or ETD	IMS	CID

Table 1. Modes of operation available with the Triwave geometry.

One particularly powerful mode of operation is Time Aligned Parallel (TAP) fragmentation, where molecules of interest are analyzed by CID/IMS/CID. This process generates first and second generation product ions with accurate mass measurement in a very rapid and sensitive manner, enabling more detailed and confidence in structural characterization.

> " [On the use of CCS values to elucidate the position of biotransformations on drug candidates] If we had to synthesise just these three metabolites, it would probably have taken several months, whereas the ion mobility LC-MS experiment and modelling calculations is probably around a week's worth of work."
>
> GORDON DEAR, GSK
> *CHEMISTRY WORLD UK*

ANALYZER GEOMETRY AND OPERATION

Triwave design

Triwave is the technology that provides ion mobility separations in the SYNAPT family of MS products, shown in Figure 6. It is comprised of three travelling wave devices that are used to guide (trap, accumulate, release, separate, and fragment) ions in a very precise and efficient manner.[3] The IMS T-Wave is the region where ion mobility separations are performed and the TRAP and TRANSFER T-Wave regions play an important role in facilitating separation by ion mobility. They can also operate as collision cells which broadens the experimental possibilities using T-Wave IMS. The Triwave design of SYNAPT G2 and G2-S MS instruments provides ion mobility resolution (of 40Ω/ΔΩ) capable of separating small or large molecular species,[4] and also ensures the high duty cycle (sensitivity) of the MS instrument is maintained. By combining Triwave with Quantitative Tof[15] (QuanTof™) Technology, the SYNAPT G2 and G2-S platforms also provide exact mass data over a wide dynamic range for ion mobility-separated ion species.

Principles of T-Wave separation

Ion mobility separation is achieved by driving ions through a neutral buffer gas with an electric field. As ions are driven forward, they interact/collide with the neutral buffer gas, which slows them down in a mobility-dependent manner, causing ions of different size, shape, charge, and mass to transit through a mobility device at different rates. To put it simply, ions with more compact structure transit more quickly through the buffer gas than ions with more open structure.

The mobility separation of ions in a drift tube (the classic form of IMS employed in research MS instrumentation[6]) and in a T-Wave ion guide both use electric fields to drive the ions through a region of gas. The classical drift tube[16] uses a uniform, static electric field/voltage gradient to separate ions, as shown in Figure 7. However, the IMS T-Wave uses non-uniform, moving electric fields/voltage pulses to separate ions, as shown in Figure 7.

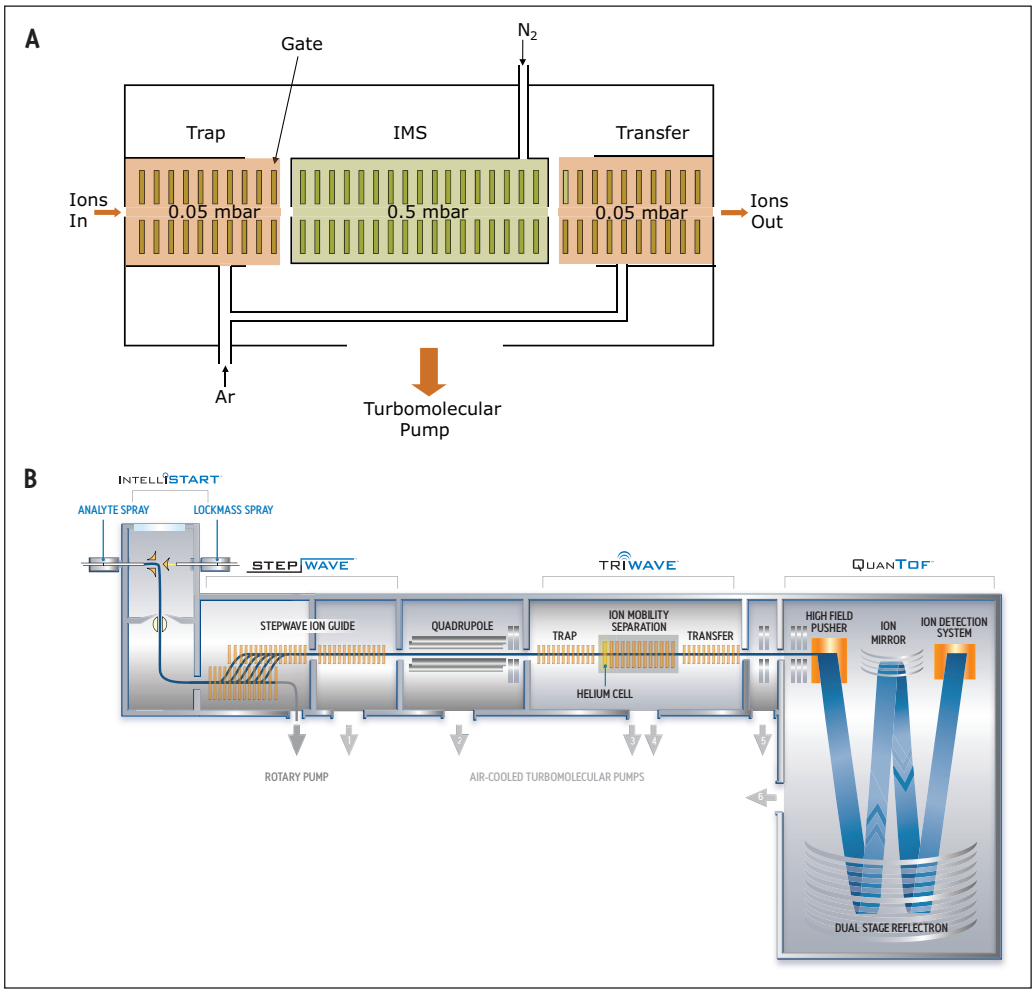

Figure 6. Schematic showing (a) the Triwave geometry, typical operating pressures and pumping configuration; (b) the SYNAPT G2-S high resolution Mass Spectrometry system, containing Triwave.

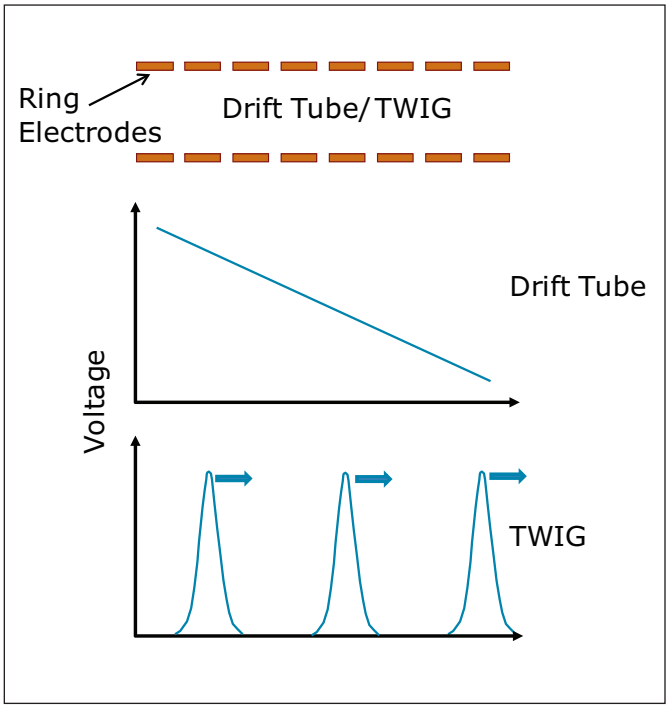

Figure 7. Electrical fields used for IM separation.

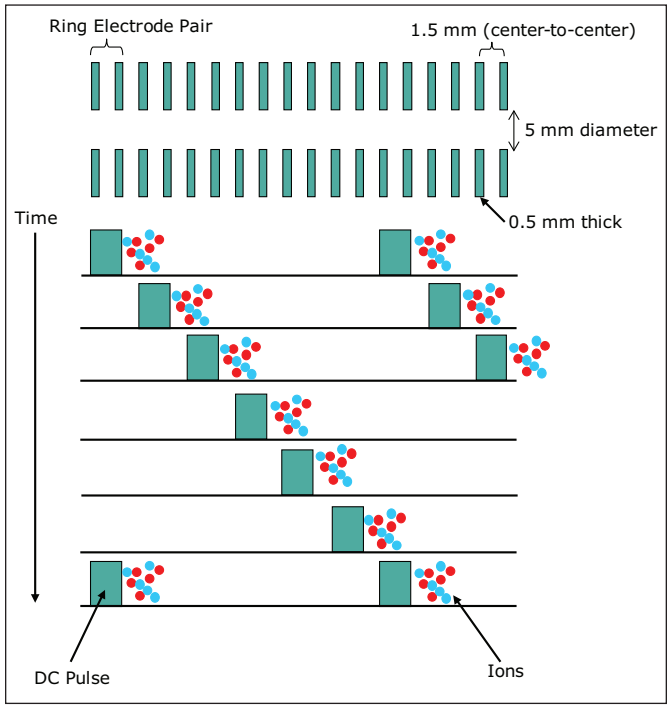

Figure 8. Operation of the T-Wave.

A T-Wave ion guide, along with typical dimensions, is shown schematically in Figure 8. Opposite phases of RF voltage are applied to adjacent electrodes to provide radial ion confinement and high transmission. The travelling wave DC voltages are applied to electrode pairs in repeating sequence along the device and step to the next adjacent electrode pair at fixed intervals. In this manner, ions are propelled along the device.

Due to relaxation of the applied DC voltage in the IMS T-Wave, the voltage profile driving the ions through is not sharp-edged, as in Figure 8, but has a smoothly varying profile, as shown in Figure 9.

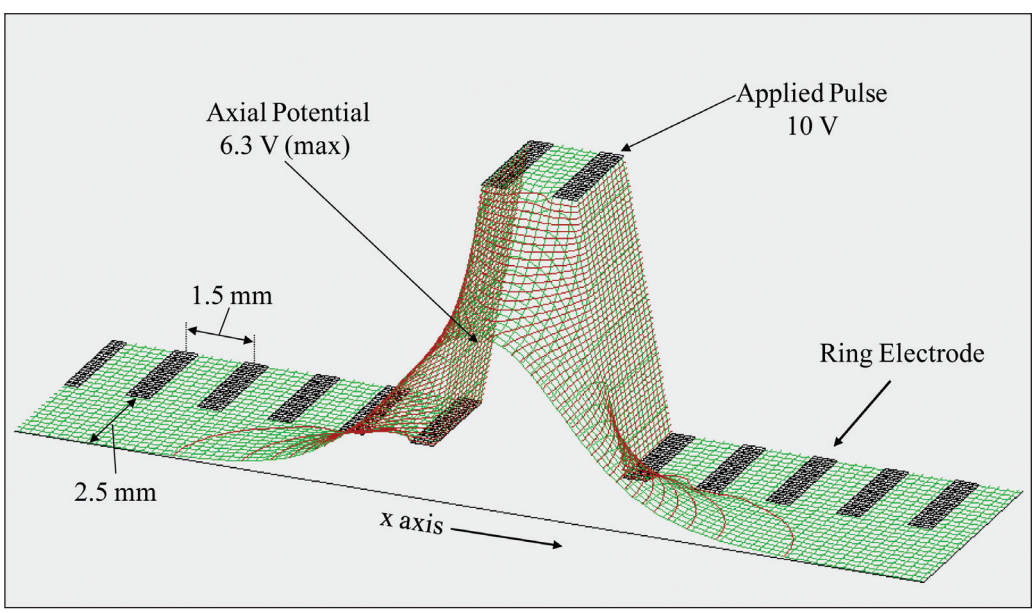

Figure 9. Profile of electrical field used in T-Wave IM separation.

Due to the high gas pressure (typically 2-5 to 3 mbar N_2) in the IMS T-Wave, the ion species experience multiple collisions (with the buffer gas molecules) as they are being propelled through by the field. The ability of an ion species to keep up with the wave depends on its mobility. Those species with high mobility surf more on the wave front and are overtaken by the wave less often than those species of low mobility, hence for a packet of ion species entering the cell, mobility separation occurs. Accordingly, ions of high mobility have shorter transit times through the device than those of low mobility.

N_2 is used as the IMS gas in most cases because it enables effective separation of molecules of all classes, while the T-Wave speed is typically the main parameter the user needs to optimize the separation. However, the extent of separation can also be improved with the use of different IM gases (*e.g.* CO_2)[17], and a range of other parameters can be utilized for more advanced analysis.

T-Wave IMS in practice

The IMS T-Wave is present in the Triwave region of all SYNAPT MS instruments. A simplified schematic of the Triwave region is shown in Figure 10.

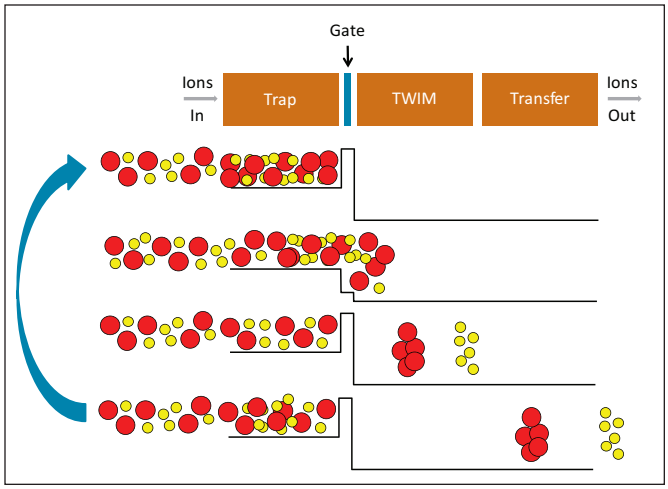

Figure 10. Conducting IM separations in the Triwave.

Ion species continually enter the TRAP T-Wave region and are prevented from exiting by a potential barrier applied at the gate; they accumulate during the previous mobility separation. There is no Travelling wave applied in the TRAP T-Wave region during ion accumulation. Once the previous mobility separation is complete, the gate is opened for a period of time (by removal of potential barrier) to admit a packet of ions into the IMS T-Wave, in which the ions then separate according to their mobility by the action of the T-Wave in the presence of the buffer gas. Once the mobility-separated ions exit the IMS T-Wave they are transported through the TRANSFER T-Wave cell with a travelling wave, which maintains the mobility separation, to the ToF analyzer. Once all of the ions have been detected in the ToF analyzer, the gating in of the next packet of ions occurs and the cycle is repeated.

This synchronized process ensures that no ions are lost during IMS in order to maintain full duty cycle of the analysis. This is in contrast to many conventional drift tube IMS instruments where there is often no trapping capability and limited ion confinement (resulting in an overall duty cycle which can be as low as 1%). The Triwave design ensures that the IMS separations are carried out with near maximal transmission, therefore providing the benefits of IMS at the limits of MS sensitivity. The helium cell at the entrance of the IMS T-Wave plays an important role by minimizing scatter or fragmentation of ions as the transit from a high vacuum to high pressure environment.[4]

The T-Waves – that drive the ions through the IMS cell – are implemented with a repeating pattern, shown in Figure 11, in order to optimize the speed and extent of the separation. Ion mobility separations are typically performed in the tens of milliseconds timeframe.

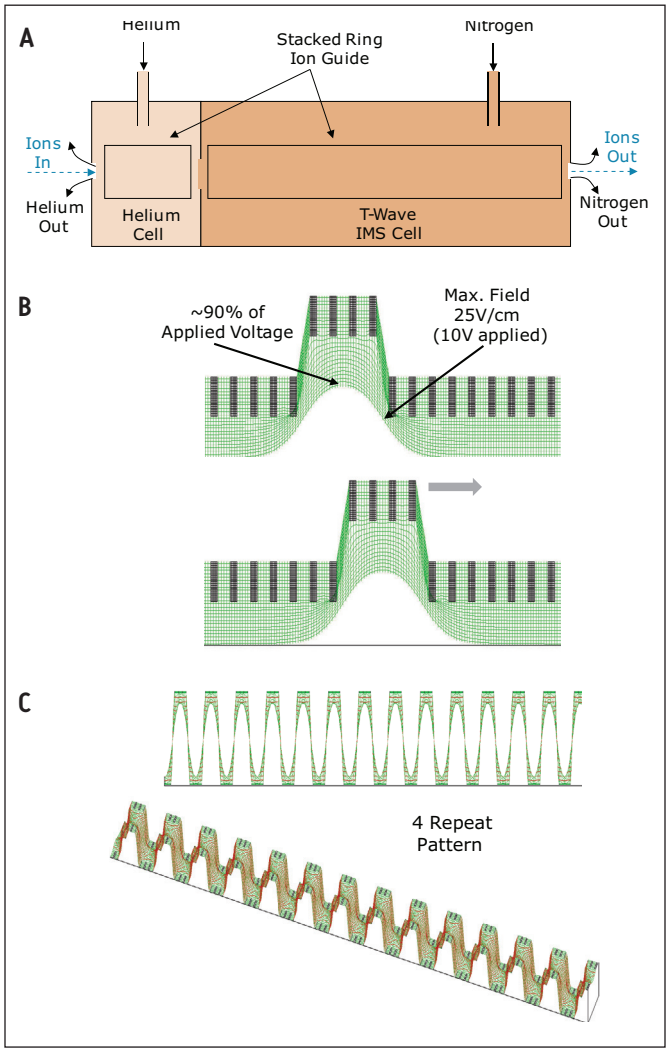

Figure 11. (a) Schematic of the IMS T-Wave. (b) The applied T-Wave DC voltage in the IMS cell of the SYNAPT G2-S steps to the next adjacent electrode pair in the ion guide and so on along the device, propelling in the direction of motion. (c) The repeat pattern of the T-Wave in the IMS cell of the SYNAPT G2-S.[4]

SUMMARY

The development of Triwave has transformed the analytical scope and capability of tandem mass spectrometry-based analysis provides a unique opportunity to extend scientific understanding and makes new discoveries possible that were not feasible with other analytical approaches. The orthogonal nature of ion mobility separations provides a significant improvement in analytical selectivity, specificity, and versatility that has been described in over 200 peer reviewed papers from small molecules to peptides and large protein complexes. The rapid and continued development of software tools to automate processing of acquired data will continue to allow more scientists from all backgrounds to access the unique power of Triwave on a routine basis. The experimental capabilities described provide organizations with a new, efficient discovery tool that can help them better compete in a wide range of industries and research disciplines.

Further Reading

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